

3. S. PALADINO, F. UGOLINI and E. B. CHAIN. — Fermenters of 90 and 300 l capacity for vortex and sparger aeration.

Summary. — Small scale pilot plant fermenters of 10, 90 and 300 l total capacity are described with full details for their construction and maintenance. The fermenters (of stainless steel) are suitable for aerobic fermentations with non-pathogenic micro-organisms. They have been specially designed for aeration by the vortex system using fast agitation but can quickly be converted for aeration by means of an air sparger in the presence or absence of baffles. The operation through a complete fermentation cycle, of a working unit comprising a seed fermenter, intermediate and fermenter proper, is described in detail. The fermenters are shown to be capable of high aeration rates and of giving trouble free service when in routine use over a long period. Sources of contamination are discussed and the necessity for reliable valves and agitation units is emphasised.

Riassunto. — Sono descritti fermentatori della capacità di 90 e 300 litri per lavorazioni in impianto pilota, riferendone tutti i particolari di costruzione e di manutenzione. I fermentatori, in acciaio inossidabile, sono adatti per fermentazioni con microorganismi non patogeni. L'aerazione può essere effettuata sia con il « sistema a vortice » che con distributore d'aria in presenza o meno di antivortici. E' descritto nei suoi particolari, un « impianto tipo » per fermentazioni a tre stadi, comprendente un « fermentatore di semina », uno « intermedio » ed uno « di produzione » e ne è stata illustrata la tecnica d'uso. Dell'« impianto tipo » è riportato lo schema di montaggio. Sono discusse le possibili cause di inquinamento.

Résumé. — La description de la station pilote de échelles du laboratoire et semi-industrielle, expose les détails de construction et d'entretien des fermentateurs de 10, 90 et 300 litres de capacité. Ces fermentateurs, en acier inoxydable, sont destinés à des fermentations aérobiques à des micro-organismes non-pathogènes. Ils ont été particulièrement conçus pour l'aération par vortex en fonction d'une forte agitation mais sont aisément convertibles au système d'aération par dispersion d'air, avec ou sans panneaux de turbulence. Les opérations, tout au long d'un cycle de fermentations, sont exposées en détail et décomposées selon les unités mises en oeuvre: fermentateur à semence, fermentateur intermédiaire et fermentateur proprement dit. On démontre

que les fermentateurs sont susceptibles de taux d'aération élevés et capables de fonctionner sans défaut même en travail de routine de longue durée.

En discutant les possibilités de contamination, on insiste sur l'importance des valves et des organes d'agitation.

Zusammenfassung. — Beschrieben werden Gärungsgefäße mit einem Fassungsvermögen von 90 und 300 l und die dazugehörigen Zwischenfermenter für Versuchsanlagen experimentellen Charakters. Einzelheiten der Konstruktion und der Routineunterhaltung werden angegeben. Die Gärungsgefäße, die aus rostfreiem Stahl hergestellt sind, eignen sich für aerobe Gärungen mit nicht-pathogenen Mikroorganismen. Die besonders für Vortexbelüftung mit schneller Rührung ohne Luftverteiler entwickelten Gefäße können sehr rasch mit Belüftungssystemen durch Luftverteiler in Gegenwart oder Abwesenheit von Prallblechen versehen werden. Die Technik eines vollständigen Fermentationscyklus, von Zwischen- zu Hauptfermenter, wird beschrieben.

Mit den verwandten Belüftungssystemen können in den Gärgefäßen hohe Sauerstoffdiffusionsgeschwindigkeiten erreicht werden. Die Gärgefäßen arbeiten mechanisch einwandfrei bei ununterbrochener Benützung über lange Zeitperioden. Quellen von Verunreinigungen durch fremde Mikroorganismen werden besprochen. Für ihre Vermeidung ist die Verwendung von zuverlässigen Ventilen und mit geeigneten Lagerungen und Stoffbüchsen versehener Rührvorrichtungen eine Hauptbedingung.

INTRODUCTION.

The present communication describes the construction of fermenters of 90 and 300 l capacity for fast agitation together with a seed fermenter, a measuring vessel for the transfer of inoculum and vessels for the sterile addition of liquids to the fermenters during fermentations (for literature on pilot plant fermenters see Discussion). Certain particulars already discussed in detail in the previous paper (CHAIN, PALADINO, UGOLINI, CALLOW and VAN DER SLUIS, 1954c) in connection with 10 l laboratory fermenters but relevant also to the present work will only be mentioned summarily below. The technique of operation for a working unit of three fermenters (seed, intermediate and fermenter proper) is described in full detail for a complete fermentation

cycle. A description of the fermentation pilot plant of which these fermenters form a part is given in another paper (CHAIN, PALADINO, UGOLINI and CALLOW, 1954b).

90 l FERMENTER.

A photograph and a drawing of a 90 l fermenter is given in plate 1 and in fig. 1. In general, all dimensions are indicated in the drawing and are not repeated in the text. Mention of dimensions in the text is only made when they are not clear from the drawing.

The fermenter consists of a jacketed body and a head, both made of stainless steel.

Fermenter head. Openings.

The fermenter head (plate 1a and fig. 2) consists of a dished stainless steel (18 Cr, 8 Ni, 0.06 C) plate, 4 mm thick with a peripheral flange (9 fig. 2, for details see 9 fig. 2-a). The flange contains 12 symmetrically drilled holes for the passage of the bolts by means of which it is fastened to the fermenter body. There are 9 holes in the fermenter head for the following purposes:

- 1) for the air inlet assembly.
- 2) for a flange serving for the introduction of the inoculum and the attachment of addition vessels.
- 3) for a flange serving for attaching the air outlet assembly.
- 4-a) and 4-b) to take two wells for the insertion of two thermometers, one for temperature regulation, the other for temperature registration.
- 5) for the introduction of the foam detecting electrode.
- 6) for a flange, to take an observation sight glass. This opening also serves for introducing the culture medium.
- 7) for a flange, to take a sight glass for the illumination of the interior of the fermenter.
- 8) for a flange to carry the bearing and stuffing box unit.

The spacing of the 9 openings is evident from fig. 2, part C.

Fittings and valves.

For all parts directly connected to the fermenters described below stainless steel pipe fittings were used throughout.

The type of valve which, after much experimentation, has proved most satisfactory for all purposes was a cylindrical plug valve with a stainless steel plunger and lining and a bronze housing. This type of valve combines several advantages which makes it particularly useful for fermentation work: 1) it has a smooth action, 2) it allows unrestricted flow through the full bore, 3) it shows a particularly high degree of resistance to wear and needs very little maintenance, in contradistinction to all other types of valves tried (globe and diaphragm valves of different construction.) This type of valve is therefore now used everywhere on the fermenters and on services directly connected with them i.e. steam, air and transfer lines.

Air inlet.

When aeration is effected by the vortex system the air enters through the stainless steel filter tube 10 fig. 2, (for details see 10-a fig. 2-b) the construction of which is very similar to that described in the preceding paper (CHAIN *et al.*, 1954c). The filter tube is connected to the fermenter head through a stainless steel piston valve as indicated in fig. 2.

A mixture of asbestos fibre (30%) and fine Corning fiberglass (70%) is used for packing, 90 gms of packing per air inlet filter.

When sparger aeration is used an air inlet filter of similar construction but larger diameter (10-b fig. 2-b), containing 350 gms fiberglass, is fitted. To the stainless steel tube entering the fermenter a stainless steel air sparger can be fitted if desired. Baffles can also be fitted if desired. These consist of three strips of stainless steel 490 mm long, 40 mm wide which can be inserted through the filling port of the fermenter and hooked on to six lugs welded to the internal wall of the fermenter body (see fig. 5). The sparger itself acts as a fourth baffle.

Air outlet.

The details of the air outlet assembly are indicated in fig. 1. The air outlet flange (3 fig. 1) carries a tee to one end of which a pressure

gauge is fitted; the air leaves through its side arm which is connected to the air outlet filter (11 fig. 1, for details see 11 fig. 2-b) via a piston valve and bend.

Bearing and stuffing box unit.

The bearing and stuffing box unit of the 90 l fermenter (fig. 3) has the same duties to perform as that of the 10 l fermenter; consequently its design and construction is similar in many details to that used for the 10 l laboratory fermenter (for discussion and description see CHAIN *et al.*, 1954c and PALADINO and UGOLINI, 1954).

The stuffing box housing (1 fig. 3, for details see 1 fig. 3-a) is turned from a stainless steel bar to approximately the same shape as the corresponding unit for the 10 l fermenter. Its dimensions are larger. Its flange is slotted in four places to take a pin spanner in order to block it to the flange (3 fig. 3-a) welded centrally in the fermenter head; the dimensions and position of the slots are indicated in 1 fig. 3-a. In addition to the four slots it contains a threaded hole into which is screwed a nipple for introducing grease for the lubrication of the lower ball bearing.

The housing of the stuffing box is externally threaded above and below the flange, it is also internally threaded at the top. The upper external thread serves for holding the knurled brass cap (5 fig. 3, for details see 5 fig. 3-a) the purpose of which is to compress the packing material. The knurled cap is held in the desired position by the locking ring (4 fig. 3). The packing material is contained in the stuffing box proper (2 fig. 3, for details see 2 fig. 3-a) which is screwed into the stuffing box housing by means of the internal thread at its top. The stuffing box proper is turned from a stainless steel bar to the cylindrical shape and dimensions indicated in 2 fig. 3-a. It is externally threaded at its top for insertion into the stuffing box housing. The packing material (packing rings with square section, of asbestos-graphite mixture, see CHAIN *et al.*, 1954c) is inserted into the interior of the stuffing box cylinder and held on an inner shoulder (2 fig. 3 and 2 fig. 3-a). The outer race of the lower ball bearing is held between the inner shoulder of the stuffing box housing and the lower end of the stuffing box cylinder (fig. 3). The stuffing box is screwed tightly into the stuffing box housing by means of a pin spanner inserted into the upper surface of the stuffing box cylinder wall (2 fig. 3-a). The inner race of the lower ball bearing is held between the inner tube of the grease cup (11 fig. 3, for details see 11 fig. 3-b) which is screwed

to the propeller shaft and the lower shoulder of the widest part of the propeller shaft (fig. 3). The thread connecting the grease cup to the propeller shaft is of fine pitch in order to ensure sufficient mechanical strength to block the inner ball race firmly in position. Greasing of the lower ball race is effected through the grease nipple in the flange of the stuffing box housing referred to above. The grease enters into a space left for this purpose below the lower shoulder of the stuffing box, through four small holes equidistantly spaced on the lower end of the stuffing box cylinder wall. The upper ball bearing is not incorporated into the stuffing box as is its counterpart in the 10 l fermenter, but is housed in a special aluminium casing (6 and 8, fig. 3, for details see 6 and 7 fig. 3-a, and 8 fig. 3-b) above the stuffing box, supported by four pillars (9 fig. 3, for details see 9 fig. 3-b) which are screwed into the internally threaded flange (3 fig. 3, for details see 3 fig. 3-a). The latter is screwed to the thread below the flange of the stuffing box housing (fig. 3). The casing is held in position on the pillars by means of four nuts and washers (14 and 15 fig. 3). The aim of this arrangement is to confer a greater degree of stability on the bearing unit which, through the type of transmission used in this case, is subjected to a lateral pull. By increasing the distance between the upper and the lower ball bearing and by housing the upper bearing in a casing of larger diameter than that of the stuffing box unit the lateral thrust for the transmission is distributed over a wider area. If the direct vertical type of transmission is used, as was done for the 10 l fermenters, it is not necessary to separate the upper and lower bearings and in this case the bearing and stuffing box unit of the 90 l fermenters can be constructed analogously to that of the 10 l fermenter with both ball bearings incorporated into the stuffing box. The outer race of the upper ball bearing is held by the two parts 6 and 8 (fig. 3) of the aluminum casing and prevented from rotating by tightening the four screws 7 (fig. 3). The inner race is a force fit on the shaft (fig. 3) just above the upper shoulder of the widest part.

Propeller shaft.

The propeller shaft of the 90 l fermenter is very similar in construction to that of the 10 l fermenter. It is a stainless steel rod (10 fig. 3-b) turned to the dimensions shown and threaded at both ends and at a point corresponding to the position immediately below the lower ball bearing. The latter thread, of fine pitch, (see above) serves for holding the grease cup (11 fig. 3) the purpose of which is to collect

excess grease. The grease cup is provided with a threaded hole closed by a screw (18 fig. 3). This serves for periodic cleaning of the grease cup (see below, under maintenance). The upper thread serves for blocking the pulley against the upper shoulder of the shaft by means of a nut and washer (12 and 13, fig. 3). The lower thread of the shaft serves for connecting the propeller which is blocked in position by means of a nut and washer (16 and 17, fig. 3).

Connection of the agitation unit to the fermenter head.

Flange 3 (fig. 3) which supports the stuffing box unit and, by means of the four pillars, the upper ball bearing, is welded into the central hole of the fermenter head (fig. 2). The welding is done on the exterior of the flange, as far removed as possible from its internal thread in order not to distort it. All parts of the bearing and stuffing box unit, including the propeller shaft, are assembled with the exception of the upper ball bearing and its casing (6 and 8, fig. 3). The fully assembled unit is then screwed into the internal thread of flange 3 against a Klingerit gasket and tightened against the fermenter head by means of a pin spanner inserted into the slots recessed for this purpose into the flange of the stuffing box housing. The aluminium casing (8 fig. 3) is then placed on the four pillars and fastened by the four nuts and washers (14 and 15, fig. 3). The upper ball race is then slipped on to the shaft and forced into place, and finally the cover plate (6, fig. 3) blocking the outer race is fastened to the aluminium casing by means of four screws (7, fig. 3).

Propeller.

Stainless steel propellers of the disk-turbine type of various diameters were used (for dimensions of a typical propeller blade see 19, fig. 3-b).

Propellers of diameters lower than that of the filling port can be attached through the latter to the propeller shaft. Larger propellers can only be fitted after detaching the fermenter head from the fermenter body.

Observation and filling port.

A stainless steel tube with a flange of mild steel into which a recess is cut is welded to the fermenter head as indicated in 6 fig. 2.

The flange, which is nickel plated, is drilled to contain four bolts welded to it. A thick plate of heat resistant glass is placed against a rubber gasket into the recess of the flange and held in position by means of a gasket and a nickel plated mild steel ring through which pass the four bolts from the flange. The ring is fastened to the flange by means of four nuts (see 6 fig. 2-a for details).

Illumination port.

The design of the illumination port (7, fig. 2) is very similar to that of the observation and filling port, but its dimensions are smaller.

Details of its construction are given in 7, fig. 2-a.

Flange for sterile additions.

A short length of stainless steel tubing with a stainless flange is welded to the fermenter head at hole 2 (fig. 2). Another stainless flange is bolted to it carrying a stainless steel piston valve. The latter is isolated by means of a steam seal. The arrangement for the connection of the addition vessels to the piston valve leading into the fermenter is illustrated in fig. 1.

Foam detecting electrode.

The arrangement for the foam detecting electrode is similar to that described by CHAIN *et al.*, (1954c) using a modified spark plug. The length of the electrode extension was 15 cm. It was screwed into a short length of stainless tubing welded to the fermenter head at hole 3 (fig. 2, for details see 3 fig. 2-a).

The same electrical circuit comprising electronic relay, automatic searcher and warning bell was used as for the 10 l laboratory fermenter.

Thermometer wells.

These are two narrow tubes of stainless steel closed at the bottom and welded to the fermenter head at holes 4-a and 4-b fig. 2 (see also 4 fig. 4).

Fermenter body.

The fermenter body (figs. 1 and 3) consists of a stainless steel cylinder approximately 90 l capacity, with a dished bottom and a mild steel flange recessed for taking a gasket (for details see 9 fig. 2-a). It is surrounded by a jacket of carbon steel (3 mm thick) welded to the body (fig. 3).

The stainless steel plate used for the body has a thickness of 3 mm and is made to withstand a working overpressure of 2 atm. It has welded to it two stainless steel 3/4" tubes with flanges, one at the centre of the bottom for draining (12 fig. 1) and one for sample taking at a position about 1/3 up from the bottom of the vessel (13 fig. 1). The drainage tube is connected through another flange to the stainless steel piston valve 14 (fig. 1) which is isolated by a steam seal. The sampling tube (13) is similarly isolated by a steam seal.

The carbon steel jacket serves for temperature control. It has three tubes with flanges welded to it, one at the bottom for draining (15 fig. 1), and two, at diametrically opposite positions about 1/3 and 2/3 of the height of the jacket, (16 and 17, fig. 1) for the water inlet and outlet. The jacket is insulated by a layer of glass wool enclosed in a sheet iron casing with an aluminium cover.

Support of the fermenter. Tightening of fermenter head on fermenter body.

In order to have the sample and drainage tubes at a convenient height, the jacketed fermenter body is supported on three legs, made of U section angle iron. These legs are bolted to three short iron pillars welded to the bottom of the fermenter body and the jacket. The fermenter head is made tight on the fermenter body by twelve stainless steel bolts and nuts passing through the flanges.

Transmission.

Transmission is effected by means of a V-belt from a 1 1/3 H.P. motor fixed on a pivoted bracket which is attached to the underside of the flange of the fermenter body by two of the bolts used for fastening the fermenter head to the fermenter body (9, fig. 2). The belt tension can be adjusted by changing the angle of the bracket by means of a bolt and two lock nuts.

300 l FERMENTER

General view.

A photograph of the 300 l fermenter is given in plate 2, a partial section in fig. 6 and a general cross section in fig. 7.

The design and construction of the 300 l fermenter is very similar in most details to that of the 90 l fermenter. As in the first section of this paper, the dimensions as a rule are given in the drawings.

Fermenter head.

The fermenter head, a dished stainless steel plate, 4 mm thick, has a peripheral flange into which are drilled symmetrically 24 holes for the bolts through which it is fastened to the fermenter body (12, fig. 8, details 12, fig. 8-a).

It contains nine openings, the same number, for the same purpose, as does the head of the 90 l fermenter. A list of the openings has been given above. The spacing of the holes is evident from fig. 8.

Air inlet and air outlet.

The arrangement for air inlet and air outlet for the 300 l fermenter is very similar to that described above for the 90 l fermenter. Details for the air inlet are given in 19 fig. 6, filter construction 19, fig. 6-a, for the air outlet in 3 fig. 9, filter construction 18, fig. 6-a. The same asbestos fiberglass mixture is used for packing; 350 gms for the inlet, 90 gms for the outlet.

As in the case of the 90 l fermenter a sparger can be fitted to the air inlet and baffle plates to the wall of the fermenter when required. (See fig. 11).

Agitation unit (Bearing and stuffing box, propeller shaft).

For the 300 l fermenter the same type of bearing and stuffing box unit was used as was employed for the 90 l fermenter. The construction of the propeller shaft is also very similar to that of the 90 l fermenter. Constructional details are given in fig. 10. (For detailed description see above and the preceding paper). The agitation unit is connected to the fermenter head in the same way as the corresponding

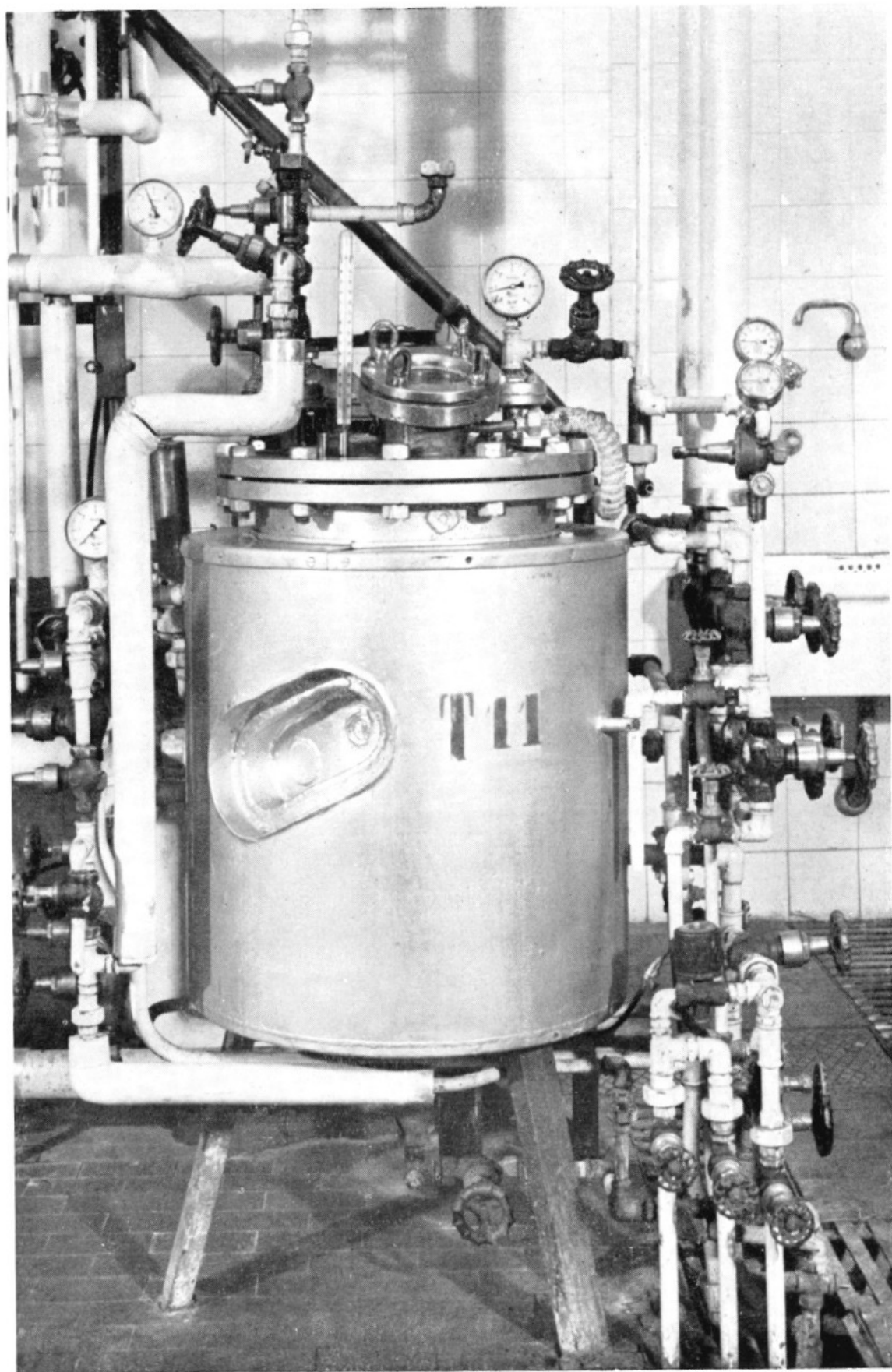


Plate 1. - 90 l fermenter; with agitation from above.

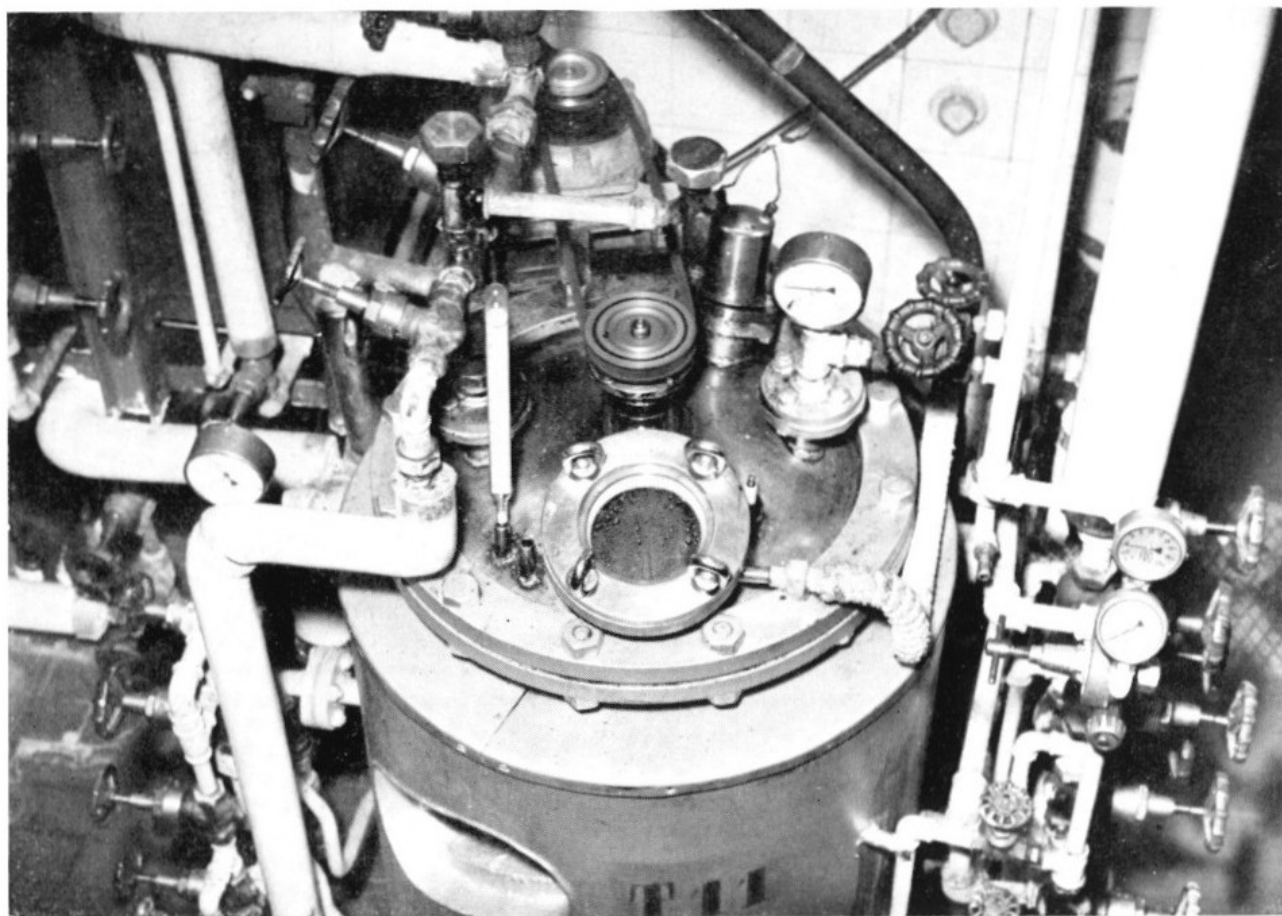


Plate 1a. - 90 l fermenter; head, seen from above.

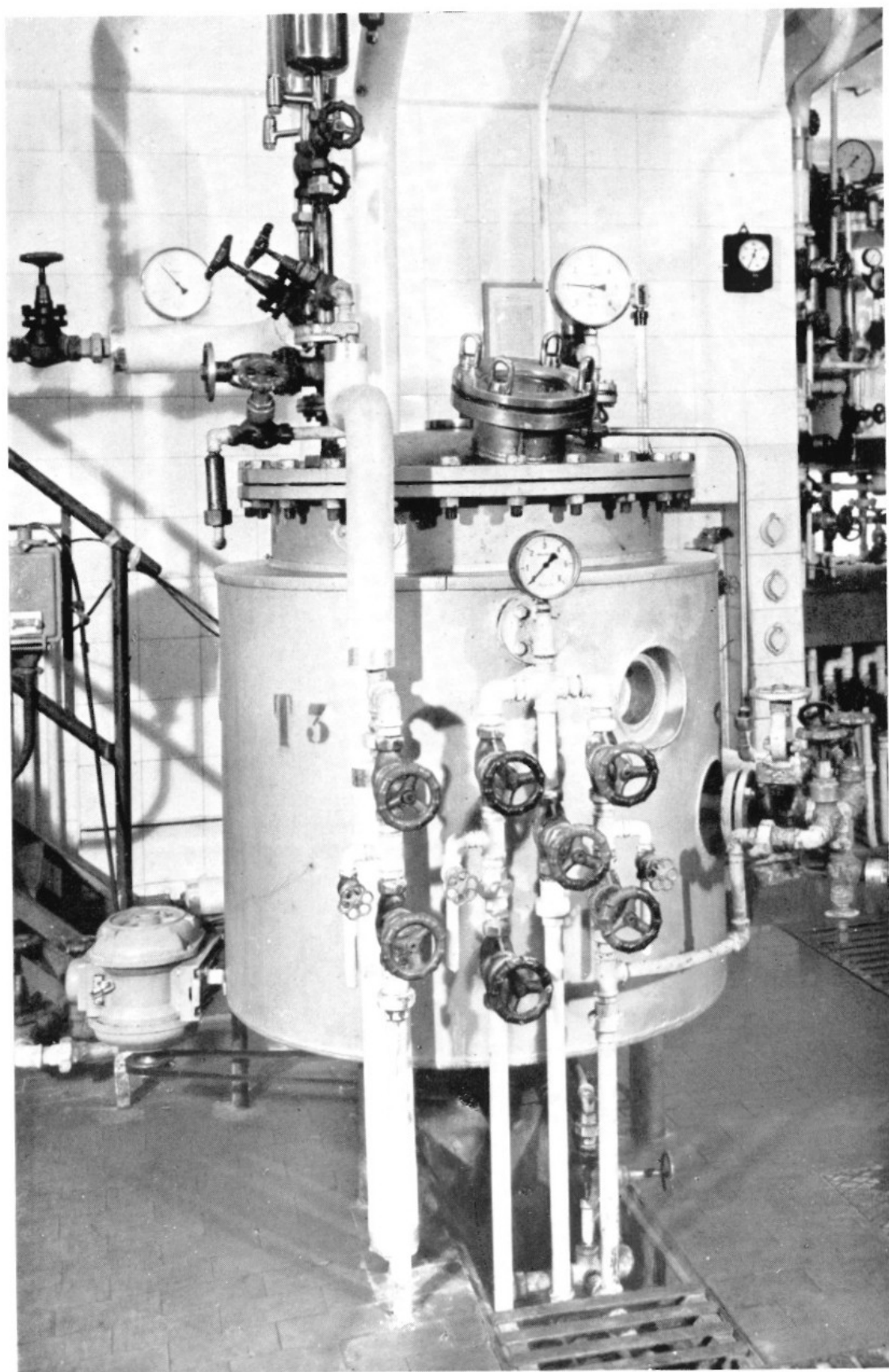


Plate 2. - 300 l fermenter; with agitation from below.

Fig. 1 - General cross section of 90 l fermenter with accessories.

- 2) Flange of addition port.
- 3) Flange carrying air outlet assembly.
- 8) Flange for insertion of agitation unit.
- 9) Flanges uniting fermenter head and body.
- 11) Air outlet filter.
- 12) Bottom outlet flange of fermenter.
- 13) Sampling outlet flange.
- 14) Bottom outlet valve of fermenter.
- 15) Bottom outlet of jacket.
- 16) Water inlet of jacket.
- 17) Water outlet of jacket.
- 18) Coupling connecting addition vessels with fermenter.
- 19) Outer cover of fermenter jacket.
- 20) Glass wool for heat insulation of fermenter jacket.

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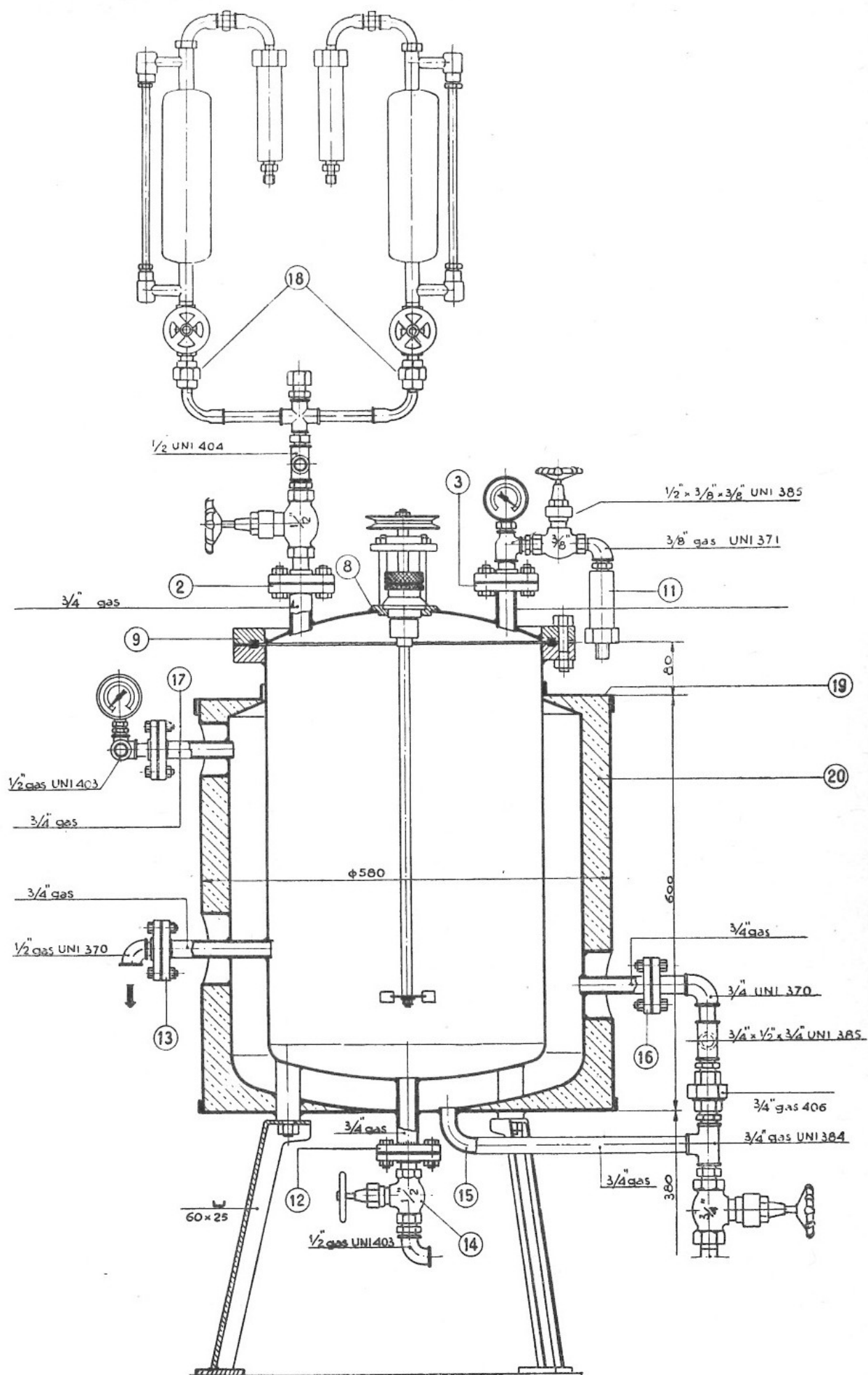


Fig. 1.

Fig. 2 - Plan of 90 l fermenter head and two cross sections.

Part A	Cross section through line MN part C.
Part B	Cross section through line PQ part C.
Part C	Plan of head showing spacing of holes.

- 1) Air inlet.
- 2) Flange of addition port.
- 3) Flange of air outlet.
- 4) (a & b) Thermometer wells.
- 5) Flange for insertion of form detecting electrode.
- 6) Observation and filling port.
- 7) Illumination port.
- 8) Flange for insertion of agitation unit.
- 9) Peripheral flanges uniting head and body.
- 10) Air inlet filter.

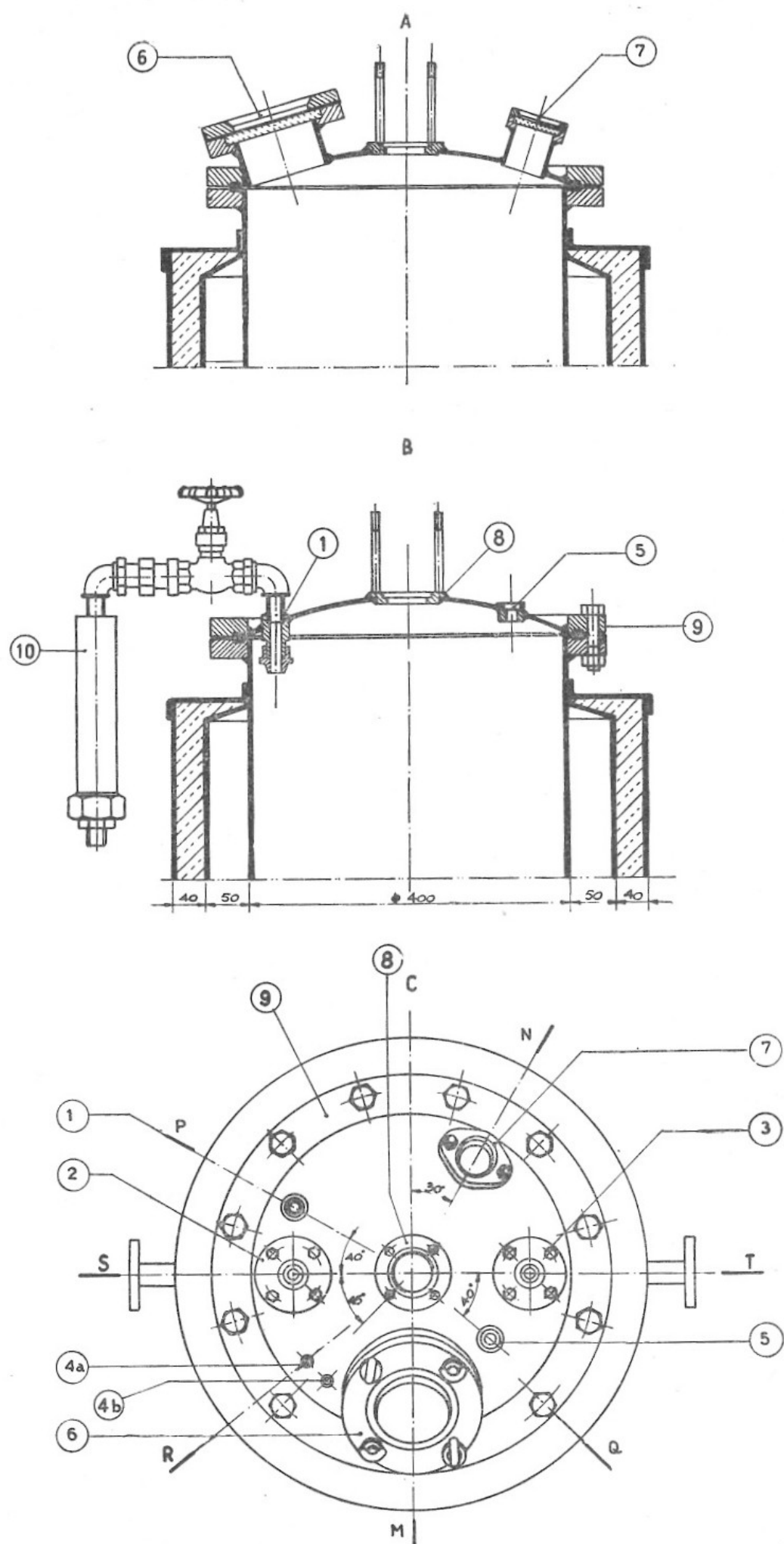


Fig. 2.

Fig. 2-a - Constructional details for 5, 6, 7 and 9 fig. 2.

- 5) Foam detecting electrode port (stainless steel).
- 6) Observation and filling port
 - d) finger nuts (4).
 - e) carbon steel flanges (2).
 - f) rubber gaskets (2).
 - g) pyrex glass window.
 - h) stainless steel trunk.
- 7) Illumination port.
 - e) carbon steel flanges (2).
 - f) rubber gaskets (2).
 - g) pyrex glass window.
 - h) stainless steel trunk.
- 9) Peripheral flanges uniting head and body
 - d) stainless steel bolts (12).
 - e) carbon steel flanges (2).
 - f) rubber gasket.

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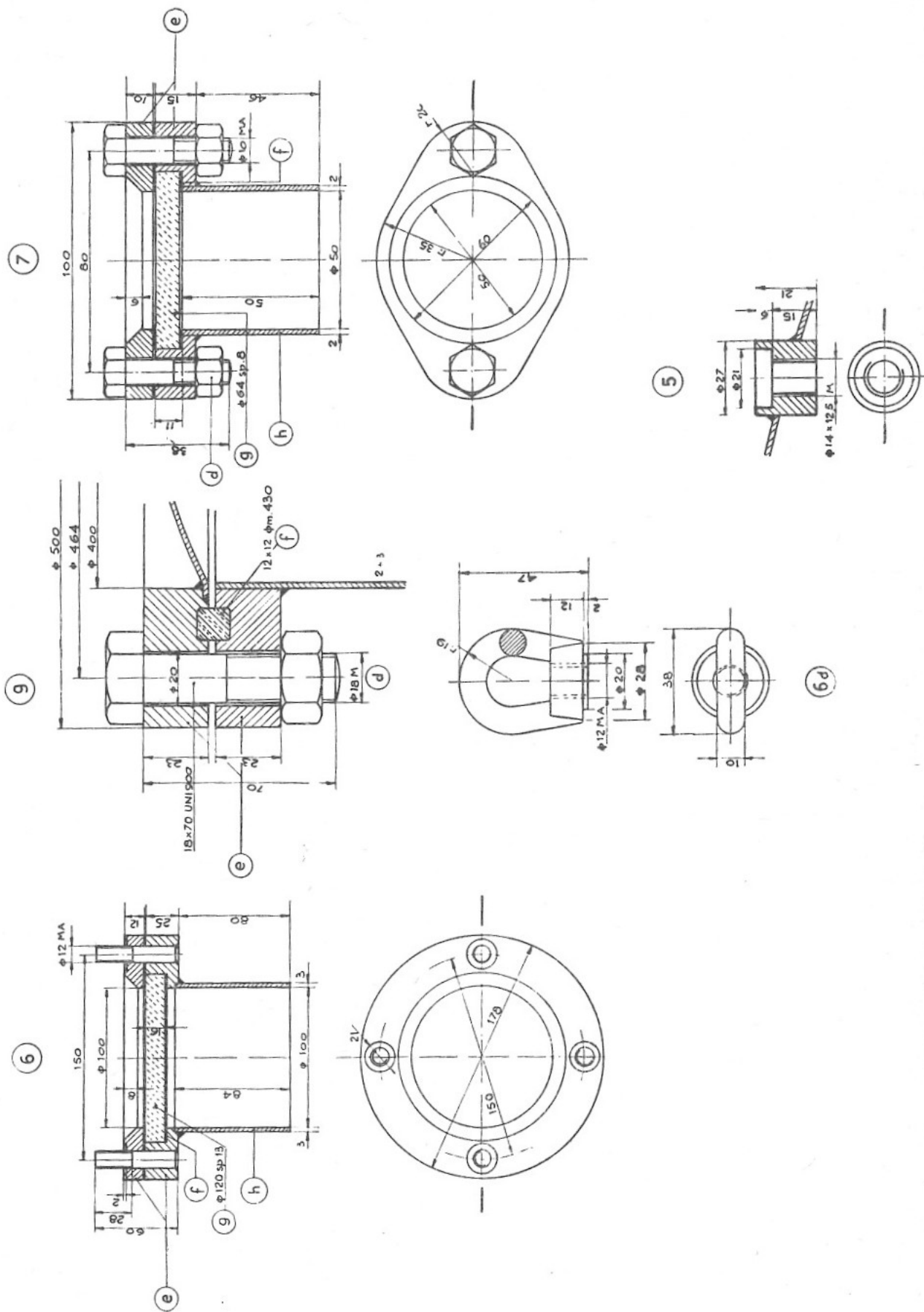


Fig. 2-a

Fig. 2-b - Constructional details of air filters for 90 l fermenters.

- 10a) Air inlet for vortex system.
 - 10b) Air inlet for baffled-sparger system.
 - 11) Air outlet for both systems.
- All parts of stainless steel.

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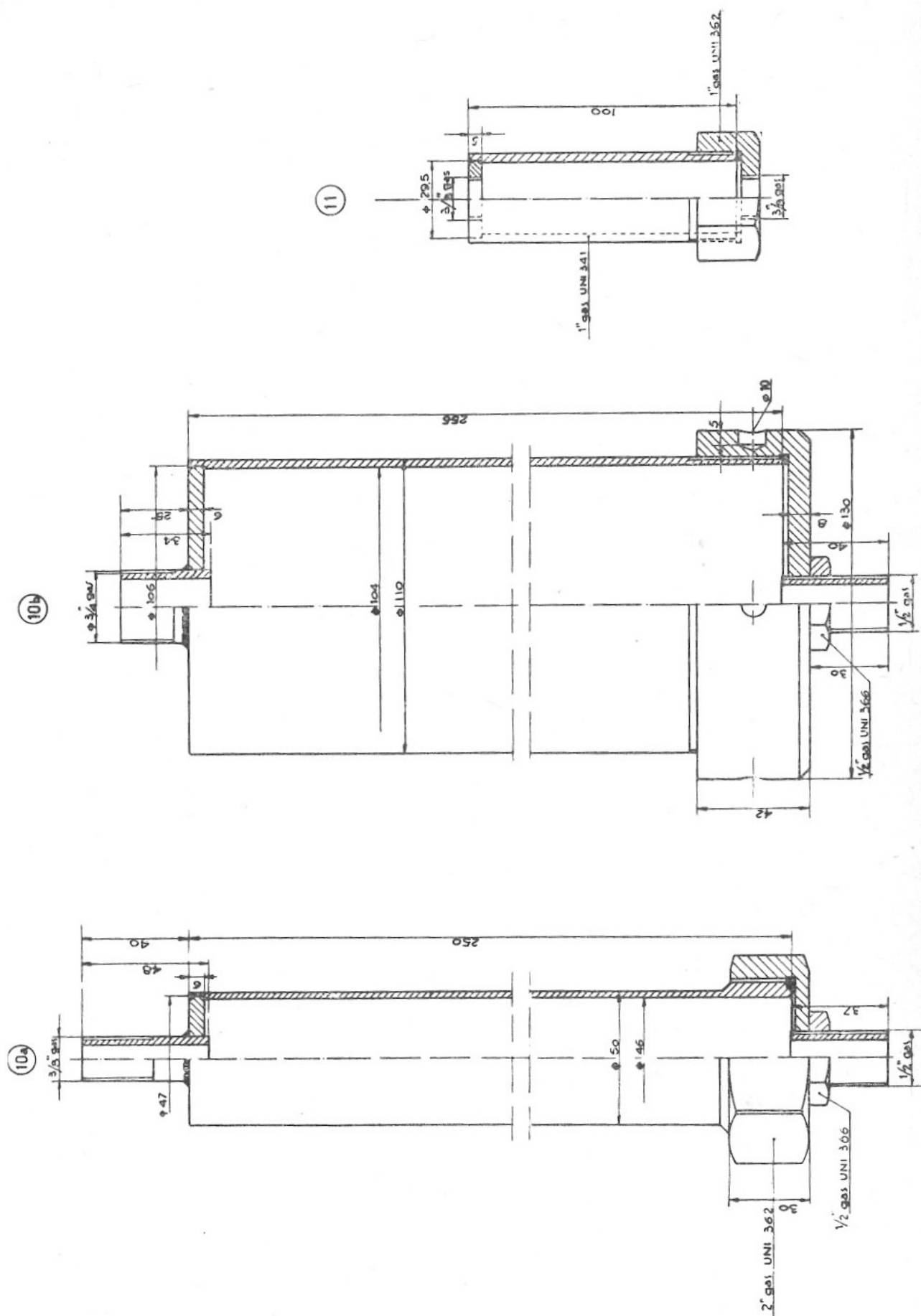


Fig. 2-b

Fig. 3 - Agitation unit for 90 l fermenters.

<i>Component</i>	<i>Material</i>
1) Stuffing box housing.	Stainless steel.
2) Stuffing box proper.	Stainless steel.
3) Flange joining agitation unit to fermenter head.	Stainless steel.
4) Lock nut.	Brass.
5) Cap regulating packing pressure.	Brass.
6) Cover plate for upper bearing.	Aluminum.
7) Screws fastening 6 to 8.	Carbon steel.
8) Housing of upper bearing.	Aluminum.
9) Supporting pillars for upper bearing.	Carbon steel.
10) Propeller shaft.	Stainless steel.
11) Grease catching cup.	Stainless steel.
12) and 13) - Nut and washer for fixing pulley to propeller shaft.	Stainless steel.
14) and 15) - Nut and washer for fixing upper bearing house to pillars.	Stainless steel.
16) and 17) - Nut and washer for fixing propeller to shaft.	Stainless steel.
18) Drain closure for grease cup.	Stainless steel.

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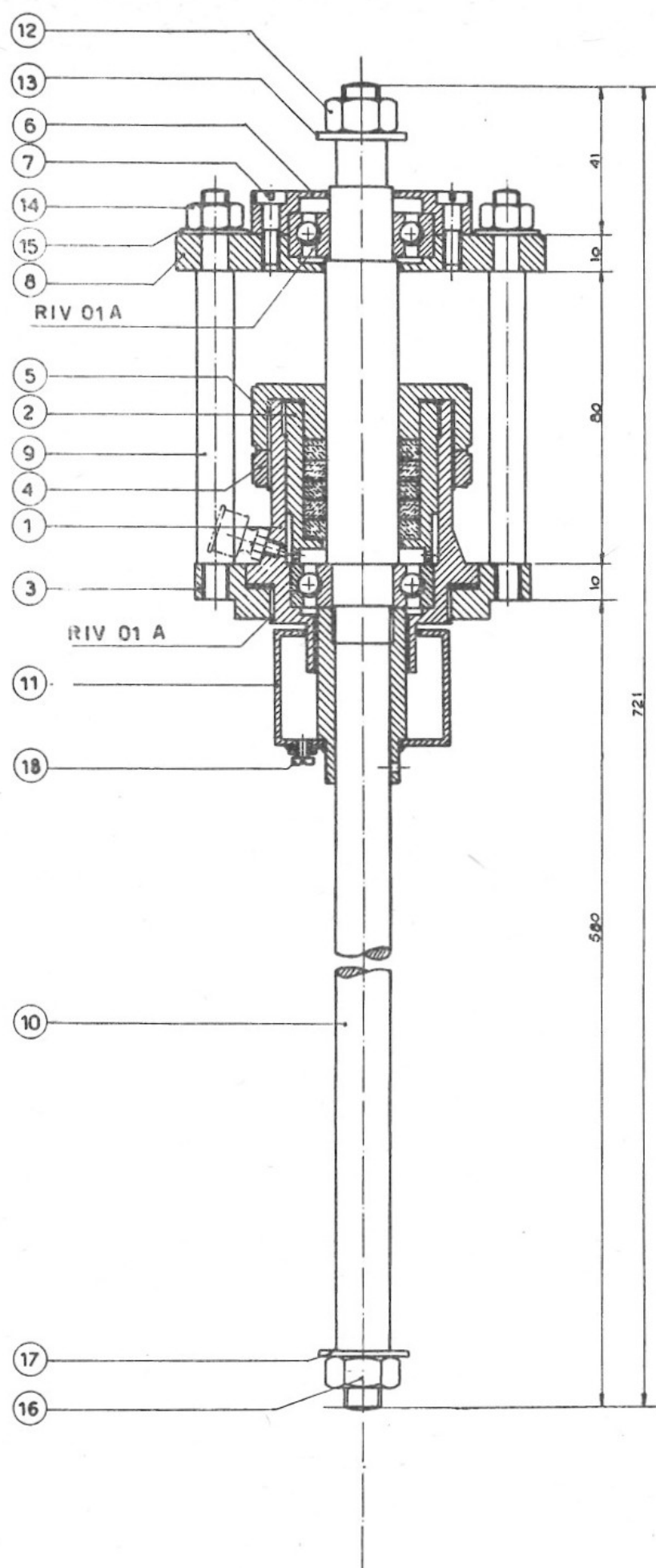


Fig. 3.

Fig. 3-a - Details of components 1-7 of agitation unit fig. 3.

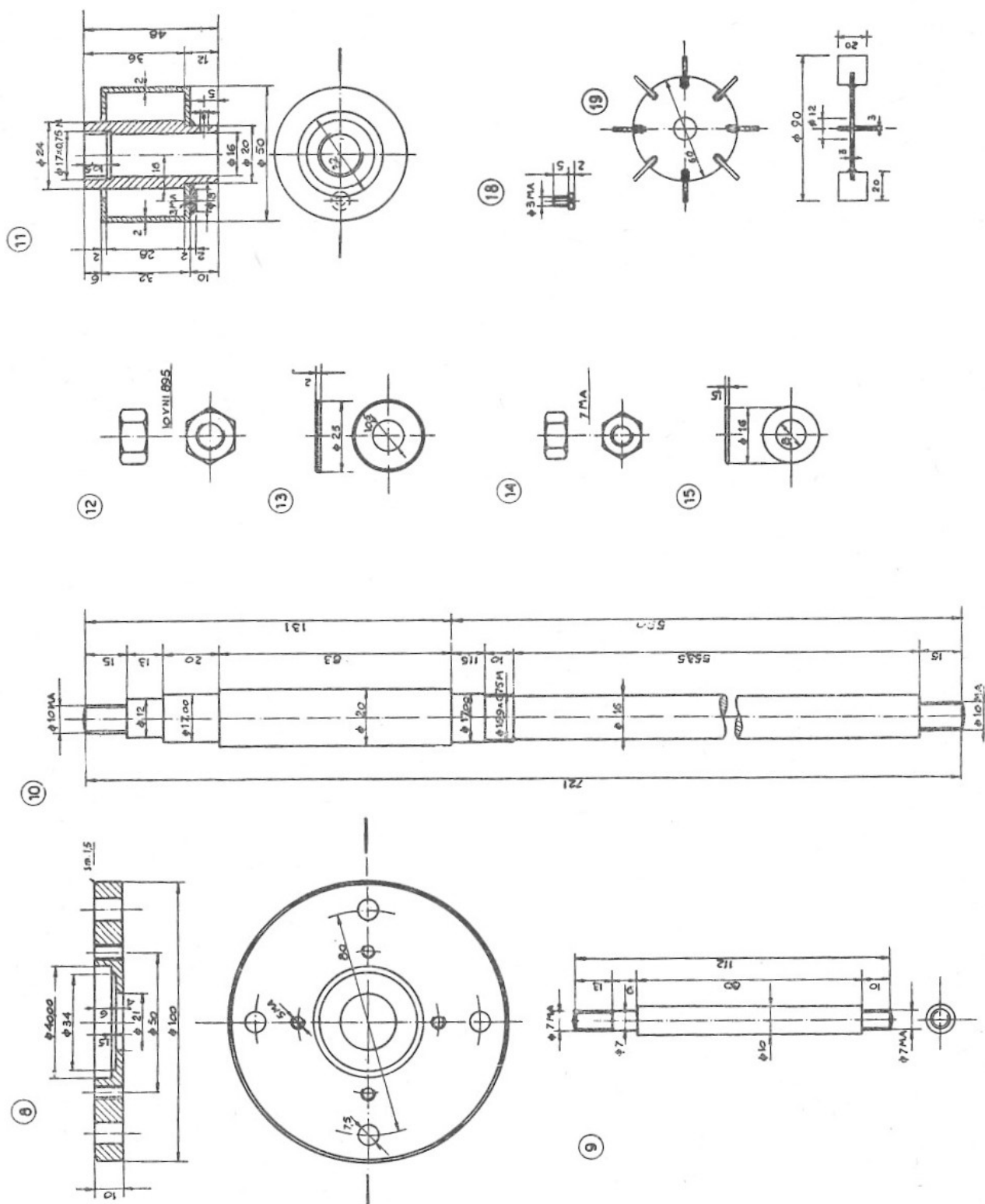


Fig. 3-b - Details of components 8-18 of agitation unit fig. 3 and propeller (19).

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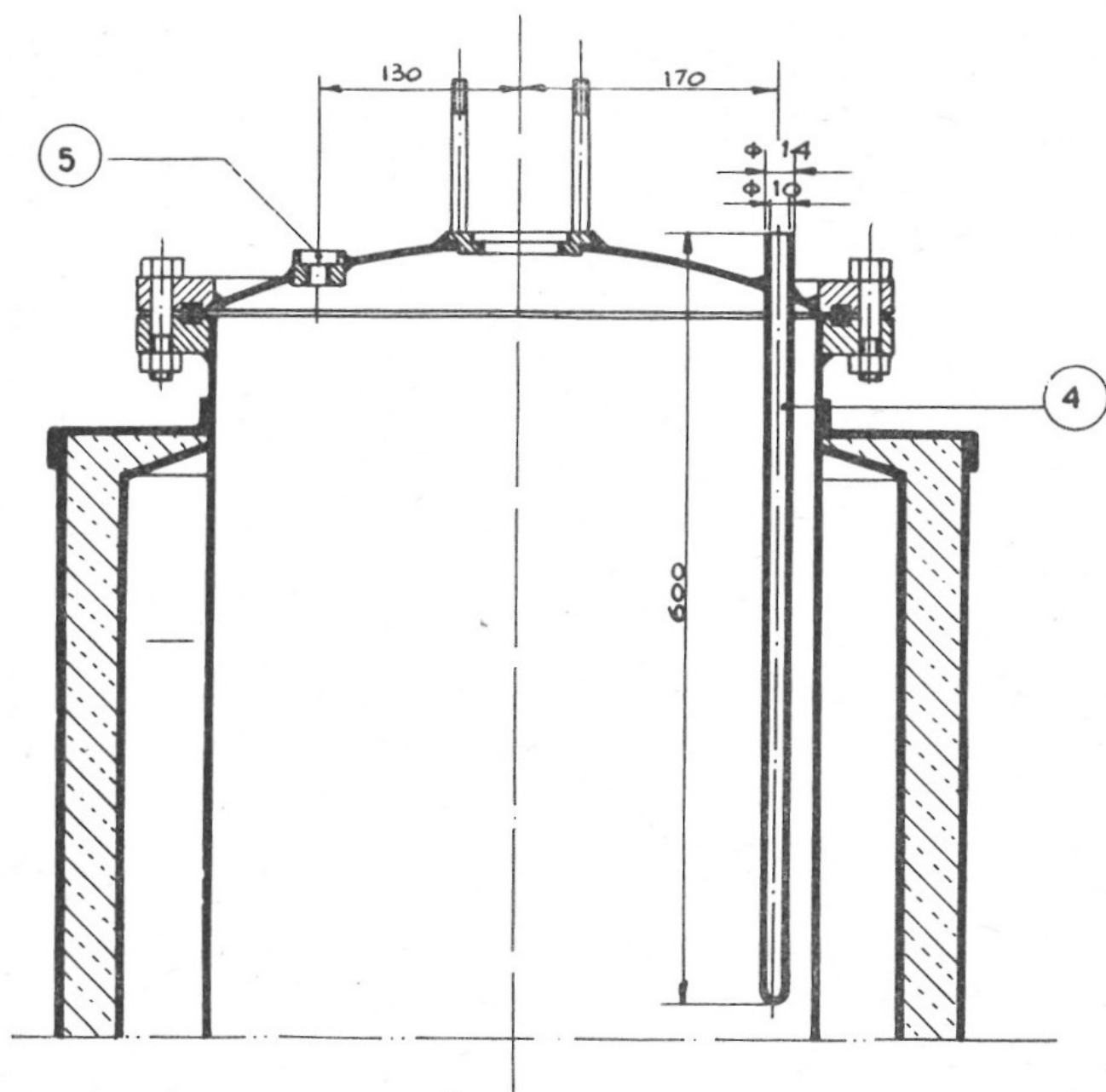


Fig. 4 - Cross section through 90 l fermenter through QR fig. 2 Part. C.

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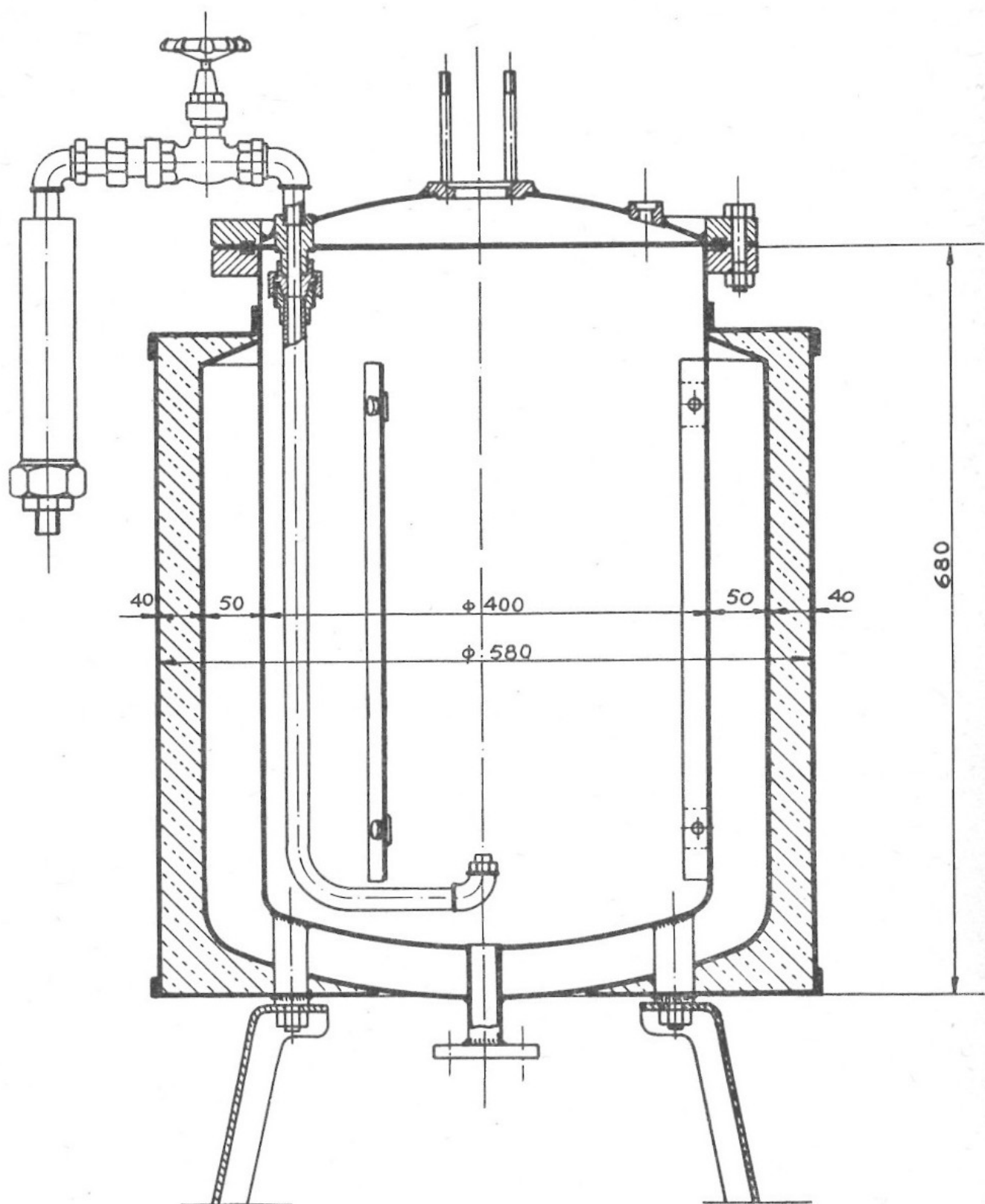


Fig. 5 - Section through 90 l fermenter showing sparger and baffles fitted.

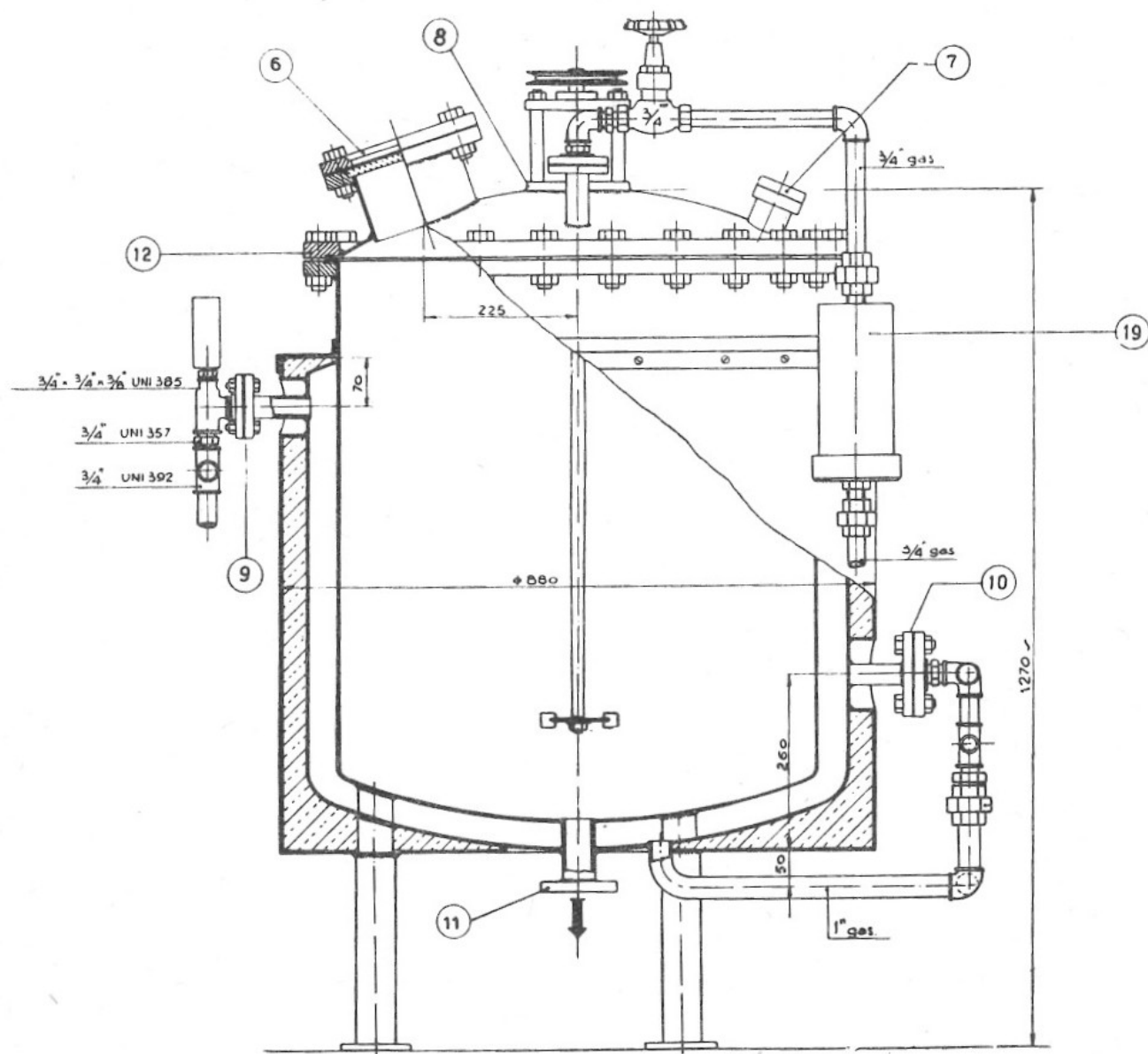


Fig. 6 - Side view (partly sectioned) of 300 l fermenter.

- 6) Observation and filling port (details in 6 Fig. 8a).
- 7) Illumination port (details in 7 Fig. 8a).
- 8) Flange carrying agitation unit (Fig. 10).
- 9) Water outlet of jacket.
- 10) Water inlet of jacket.
- 11) Bottom outlet flange of fermenter.
- 12) Flanges uniting fermenter head and body.
- 19) Air inlet filter (details in 19 Fig. 6a).

(19)

Technical drawing of a mechanical assembly, labeled (19), showing a cross-section of a cylindrical component. The drawing includes the following dimensions and labels:

- Top flange: $\phi 3/4$ gas, $\phi 106$, 34, 25, 6.
- Main body: $\phi 104$, $\phi 110$.
- Bottom flange: $\phi 130$, 40, 6, 5.
- Bottom connection: $1/2$ gas UNI 366, 30, $1/2$ gas.
- Overall height: 255.
- Overall width: 472.

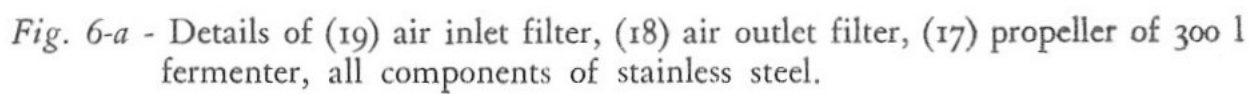


Fig. 6-a - Details of (19) air inlet filter, (18) air outlet filter, (17) propeller of 300 l fermenter, all components of stainless steel.

Fig. 7 - General section of 300 l fermenter with addition vessels in place.

- 1) Air inlet.
- 2) Addition port.
- 11) Bottom outlet flange of fermenter.
- 12) Flanges uniting fermenter head and body.
- 14) Couplings connecting addition vessels with fermenter.
- 15a) Outer cover (of sheet iron).
- 15b) Lid of cover (of aluminium, in two parts).
- 16) Glass wool for heat insulation.

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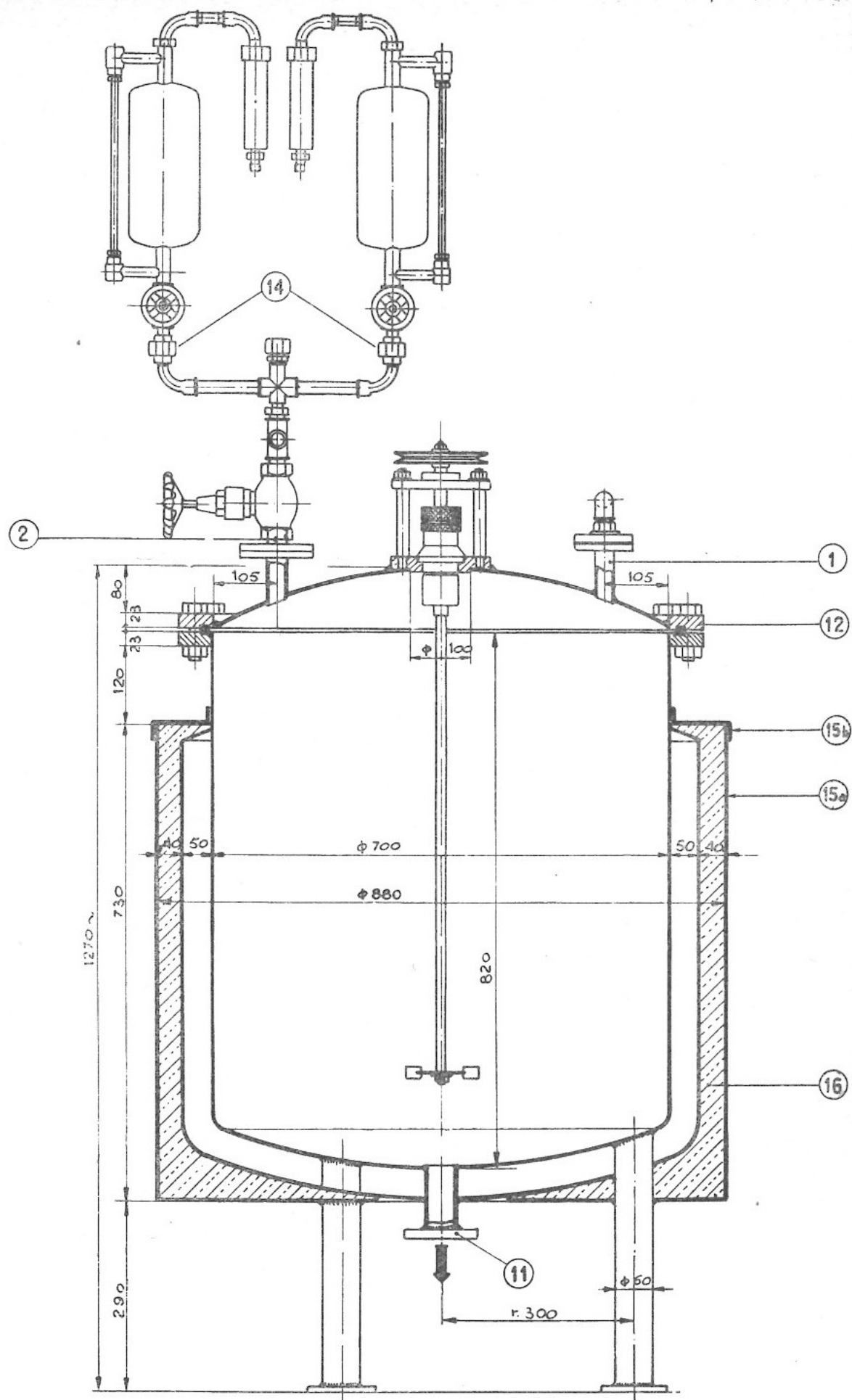


Fig. 7.

Fig. 8 - Plan of 300 l fermenter head showing spacing of holes.

- 1) Flange carrying air inlet filter assembly.
- 2) Flange of addition port.
- 3) Air outlet.
- 4) Thermometer wells (2).
- 5) Flange for insertion of foam detecting electrode.
- 6) Observation and filling port.
- 7) Illumination port.
- 8) Flange for insertion of agitation unit.
- 9) Water outlet of jacket.
- 10) Water inlet of jacket.
- 12) Flange uniting fermenter head with fermenter body.
- 13) Sampling outlet flange.

«Fermenters of 90 and 300 l capacity for vortex and sparger aeration».

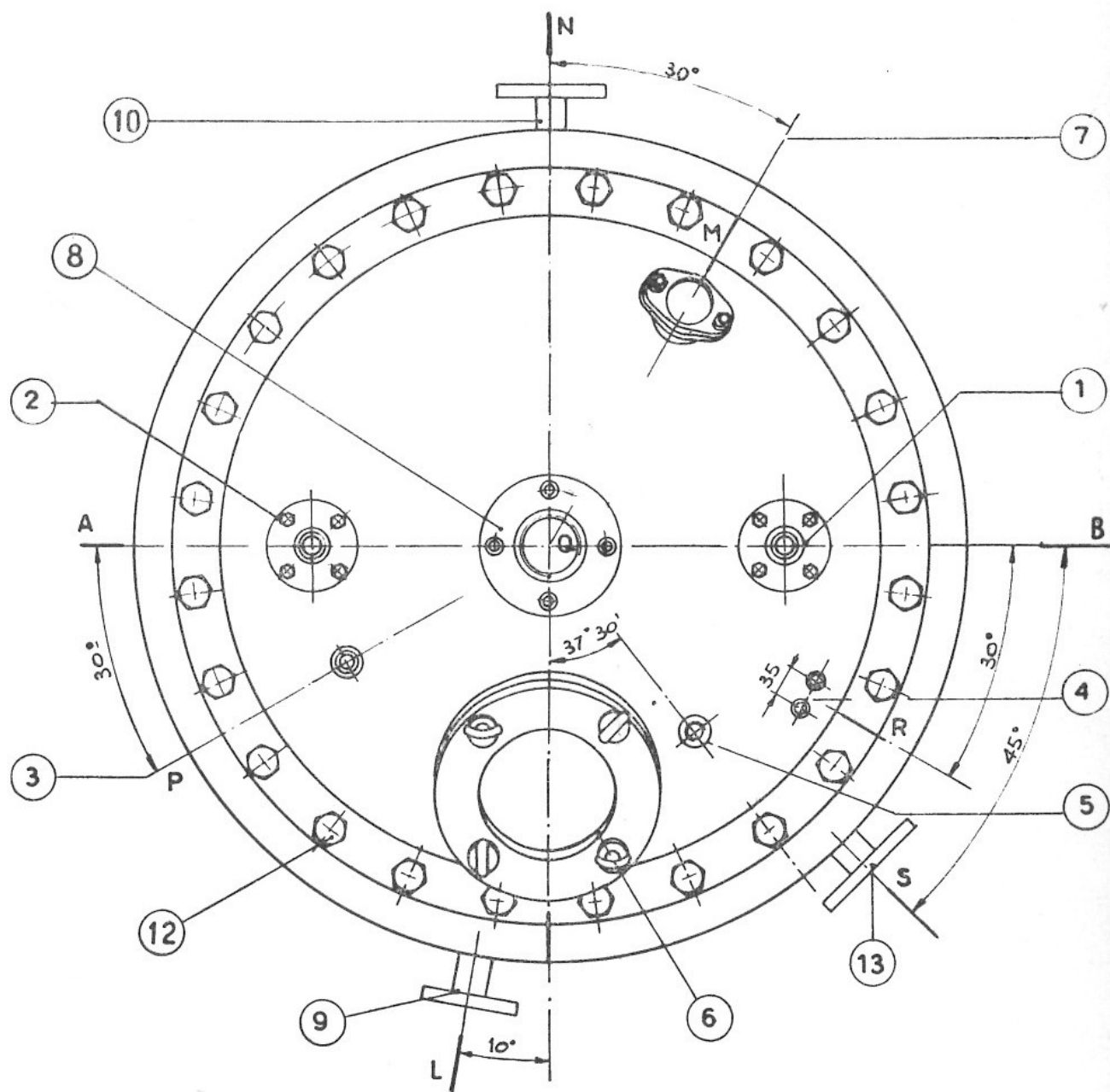


Fig. 8.

Fig. 8-a - Constructional details for 2, 5, 6, 7 and 12, Fig. 8.

- 2) Addition port flanges (stainless steel).
- 5) Foam detecting electrode port (stainless steel).
- 6) Observation and filling port.
 - e) carbon steel flanges (2).
 - f) rubber gaskets (2).
 - g) pyrex glass window.
 - h) stainless steel trunk.
- 7) Illumination port (e, f, g, h, as above).
- 12) Flanges uniting head and body
 - d) stainless steel bolts (24).
 - e) carbon steel flanges (2).
 - f) rubber gaskets.

«Fermenters of 90 and 300 l capacity for vortex and sparger aeration».

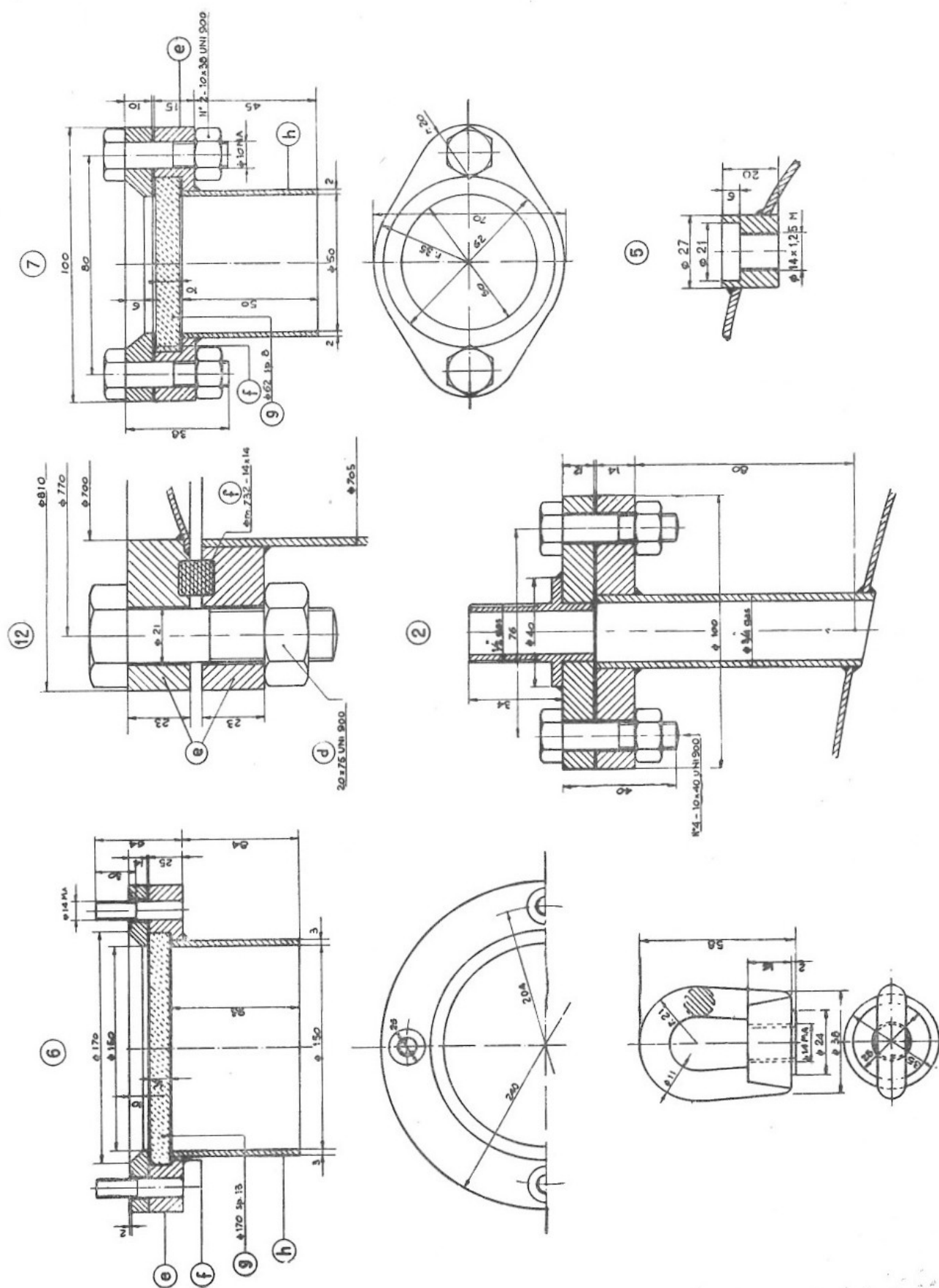


Fig. 8-a

Fig. 9 - Section through 300 l fermenter showing air outlet and thermometer well.

- 3) Air outlet.
- 4) Thermometer pocket.
- 13) Sampling outlet flange.
- 18) Air outlet filter.

«Fermenters of 90 and 300 l capacity for vortex and sparger aeration».

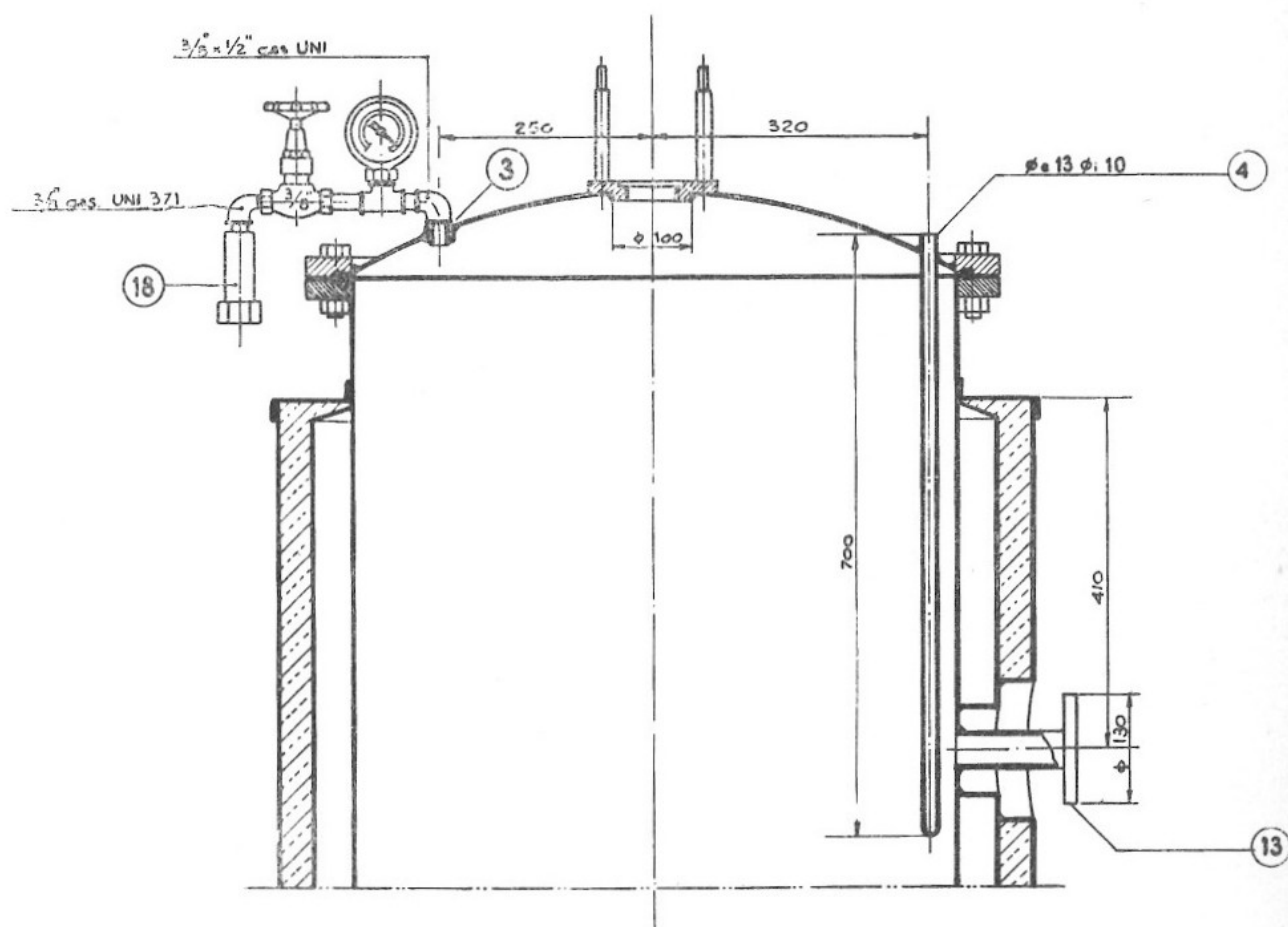


Fig. 9.

Fig. 10 - Agitation unit of 300 l fermenter.

<i>Component</i>	<i>Material</i>
1) Flange joining agitation unit to fermenter head.	Stainless steel.
2) Stuffing box proper.	Stainless steel.
3) Stuffing box housing.	Stainless steel.
4) Cap regulating packing pressure.	Brass.
5) Lock ring.	Brass.
6) Housing of upper bearing.	Aluminium.
7) Cover plate of upper bearing.	Aluminium.
8) Screws fastening 6 to 7.	Carbon steel.
9) Propeller shaft.	Stainless steel.
10) Grease catching cup.	Stainless steel.
11) Supporting pillars for upper bearing.	Carbon steel.
12) and 13) Nut and washer for fixing upper bearing housing to pillars.	Stainless steel.
14) and 15) Nut and washer for fixing propeller to shaft.	Stainless steel.
16) Drain closure for grease cup.	Stainless steel.

«Fermenters of 90 and 300 l capacity for vortex and sparger aeration».

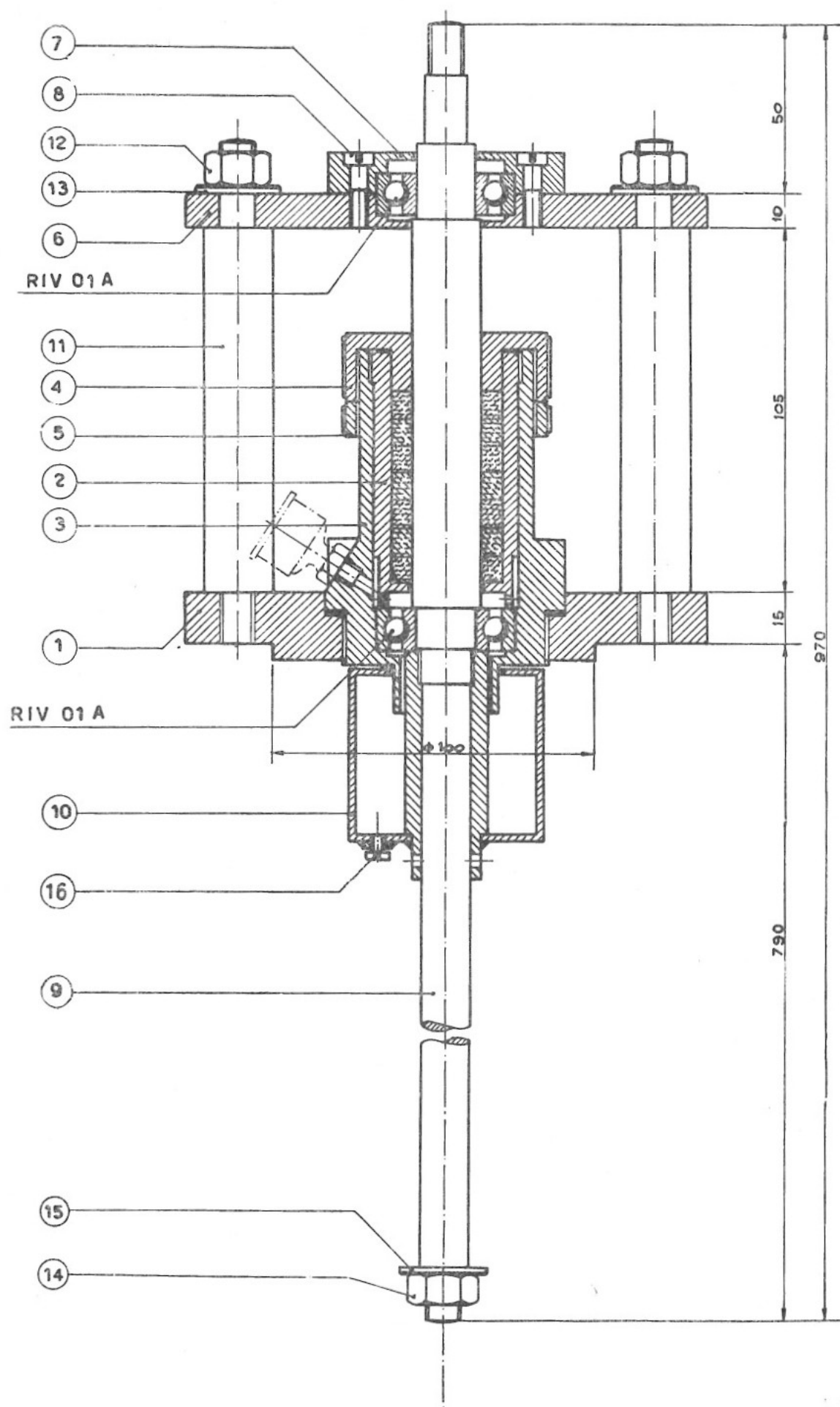


Fig. 10.

« Fermenters of 90 and 300 l capacity for vortex and sparger aeration ».

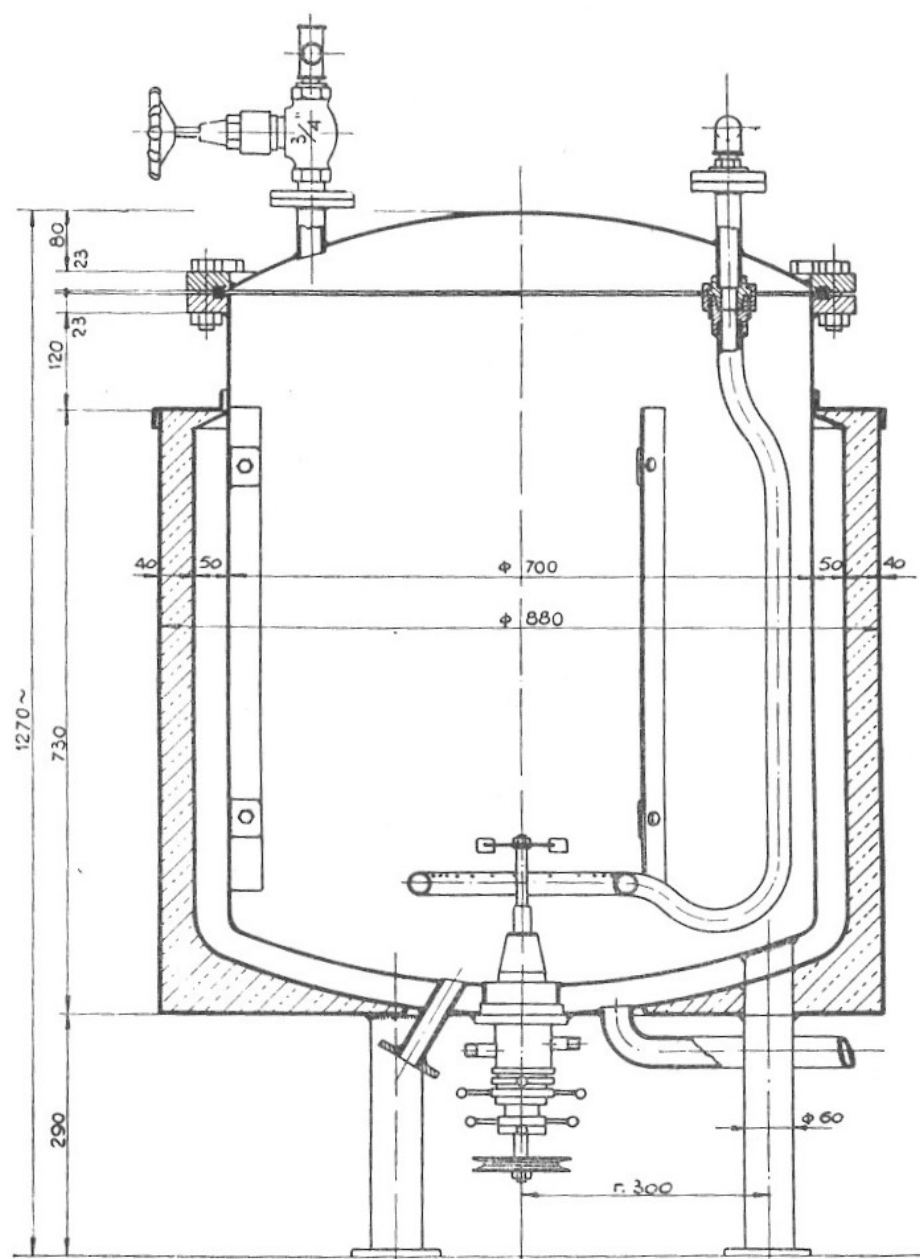


Fig. 11 - Section through 300 l fermenter with bottom entry agitation unit and with baffles and sparger fitted.

« Fermenters of 90 and 300 l capacity for vortex and sparger aeration ».

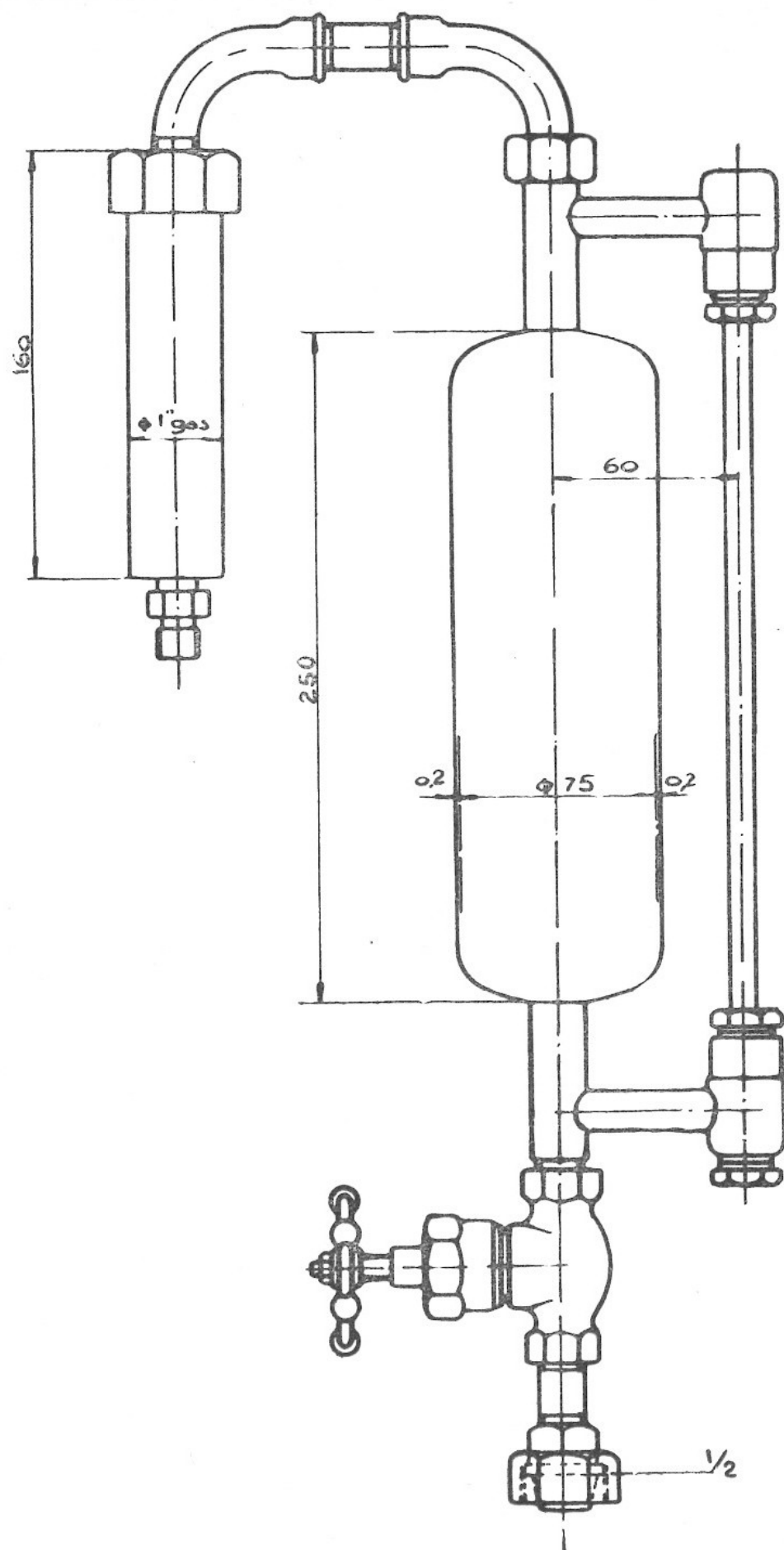


Fig. 12 - Addition vessel.

Fig. 13 - General section through seed fermenter.

- 1) Air inlet.
- 5) Inoculation port.
- 7) Water exit from jacket.
- 8) Water entry to jacket.
- 9) Bottom outlet of fermenter.
- 12) Flanges uniting head and body.
- 14) Air inlet filter.
- 16) Glass wool for heat insulation.
- 17) Stainless steel body and jacket 2 mm thick.
- 18) Sheet iron 1 mm thick.
- 19) Base plate (carbon steel).
- 20) Propeller.

« Fermenters of 90 and 300 l capacity for vortex and sparger aeration ».

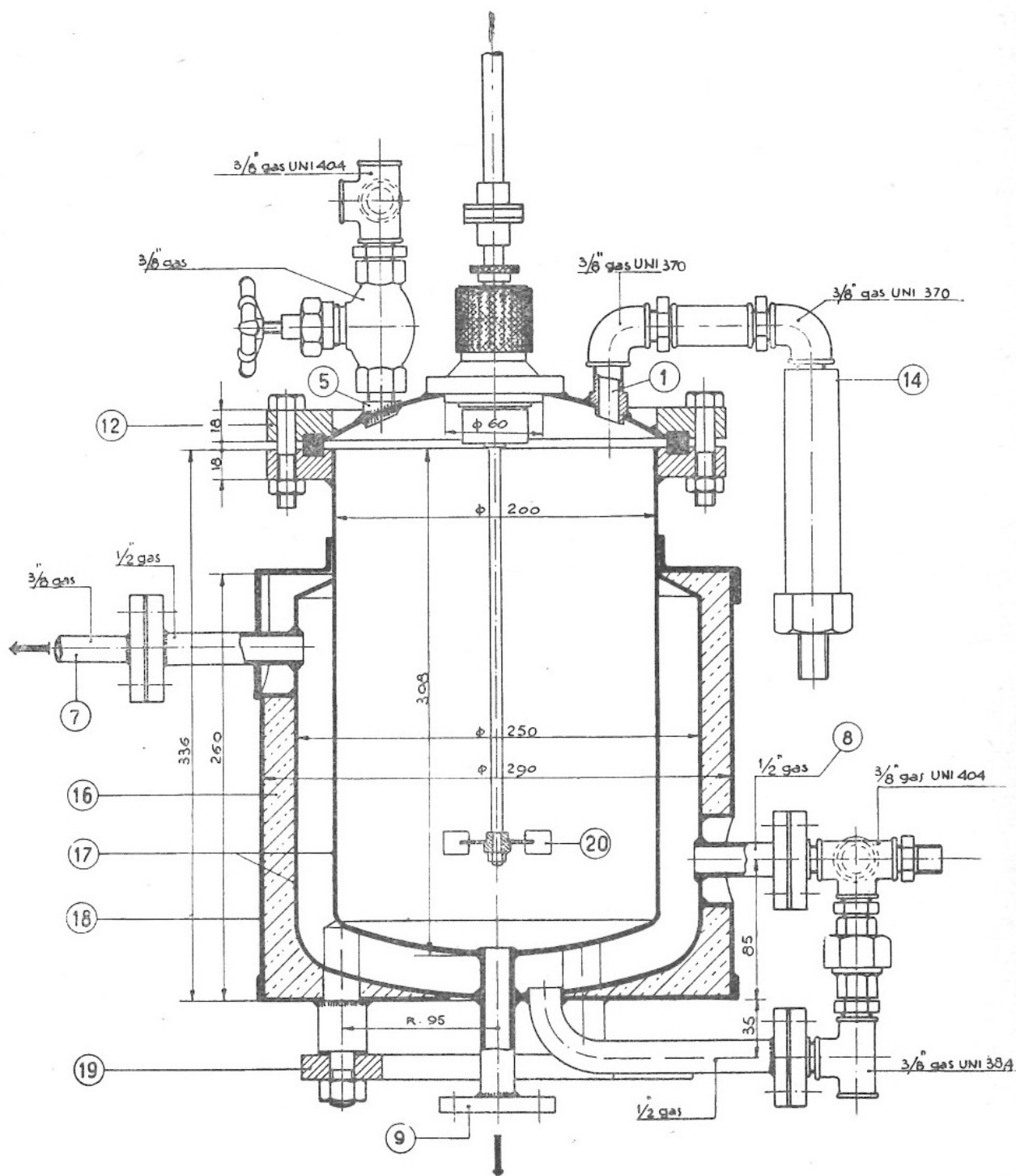


Fig. 13.

Fig. 14 - Plan of seed fermenter head and section through fermenter.

- 1) Air inlet.
- 2) Foam detecting electrode port.
- 3) Observation and filling port.
- 4) Air outlet.
- 5) Inoculation port.
- 6) Illumination port.
- 7) Water outlet of jacket.
- 8) Water inlet of jacket.
- 9) Bottom outlet of fermenter.
- 10) Sampling outlet.
- 11) Thermometer wells (2).
- 12) Flange uniting head and body.
- 13) Flange for insertion of agitation unit.
- 15) Air outlet filter.

«Fermenters of 90 and 300 l capacity for vortex and sparger aeration».

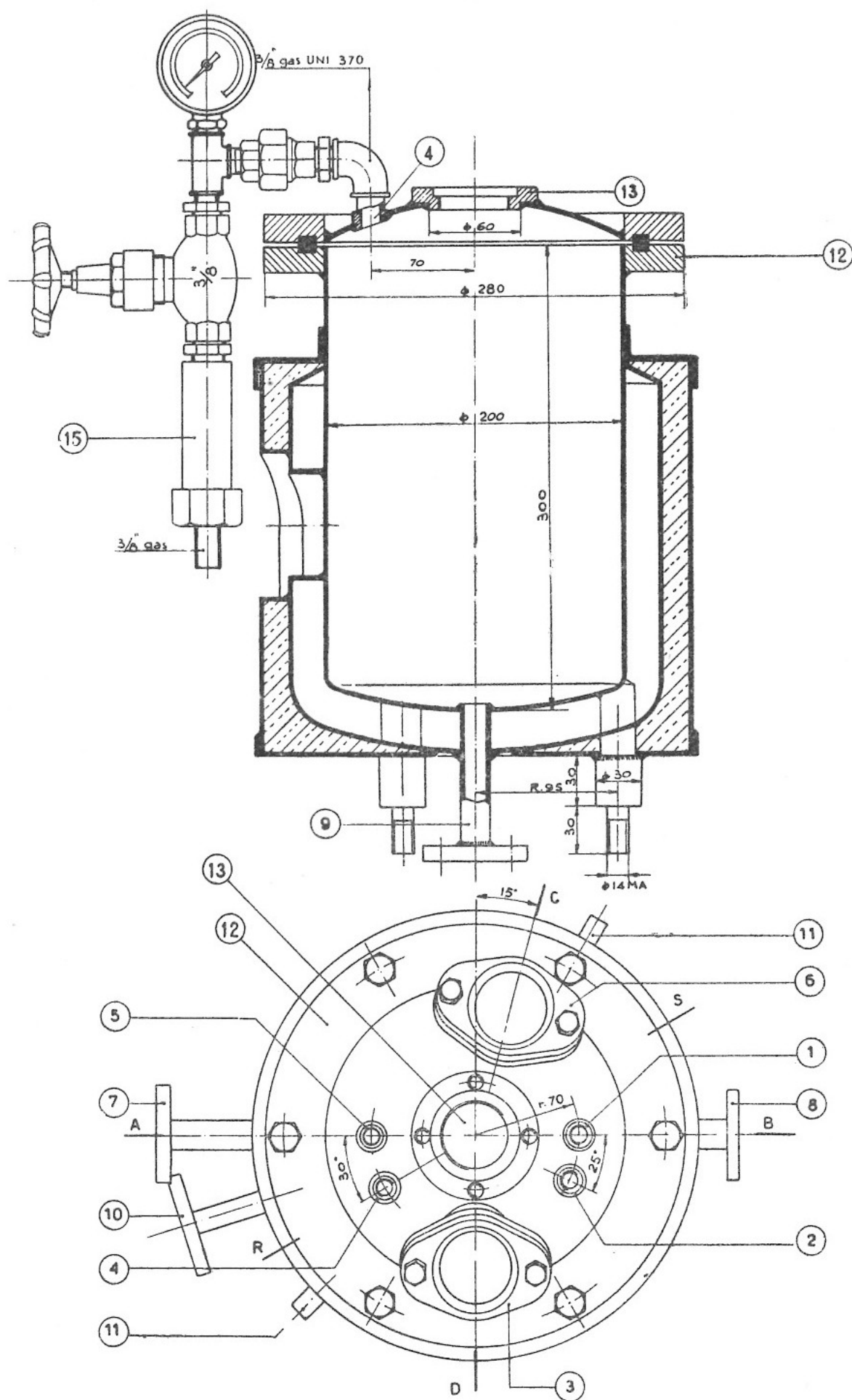


Fig. 14.

Fig. 15 - Detail of (3) Observation and (6) Illumination ports (10) Sampling outlet and (11) Thermometer well of seed fermenter.

« Fermenters of 90 and 300 l capacity for vortex and sparger aeration ».

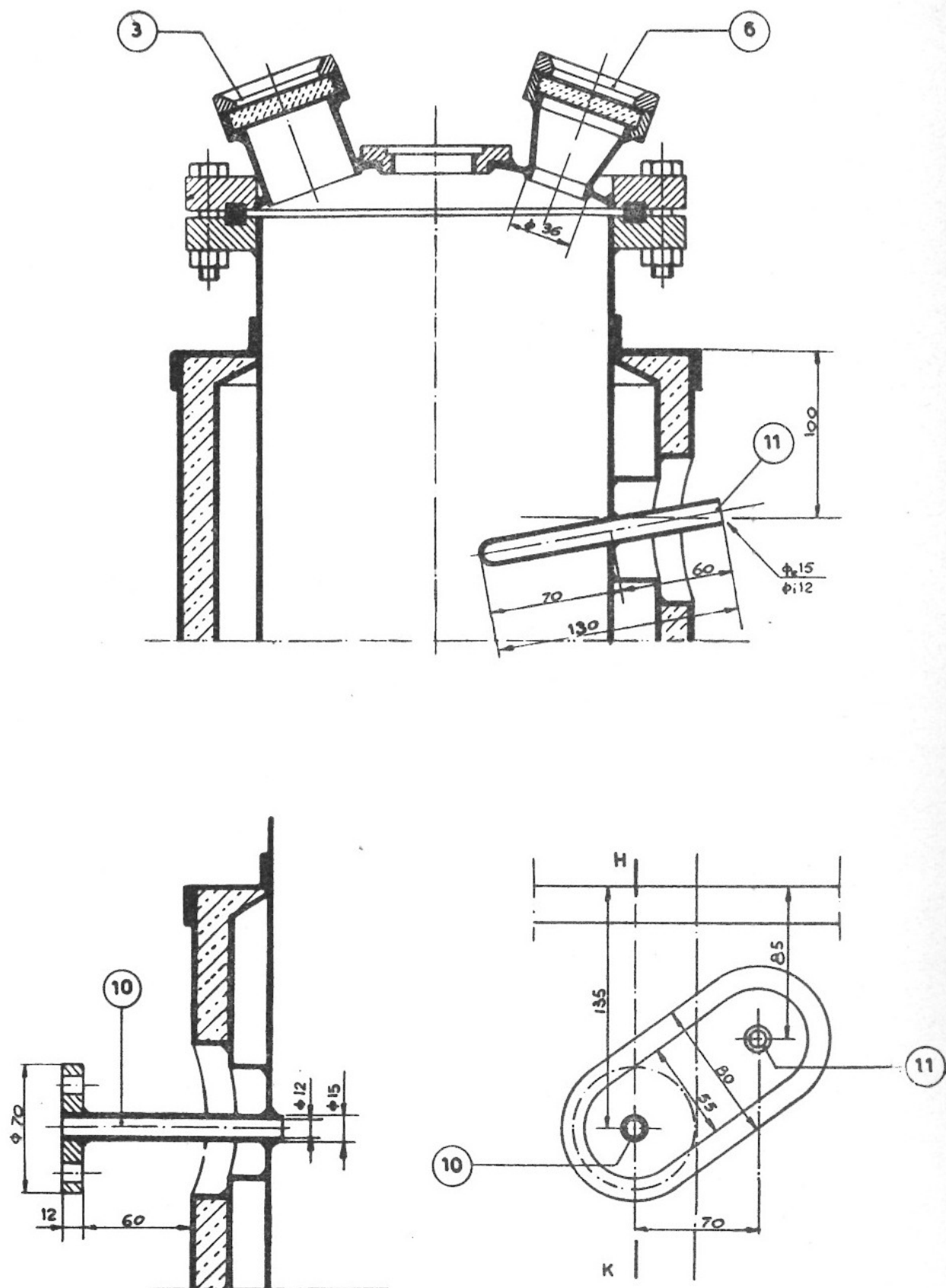


Fig. 15.

Fig. 15-a - Construction details of 3 and 6, Figs 14 and 15, and of 12, 19 and 20
Fig. 13.

- 3) Observation and filling port
- d) stainless steel bolts (2).
- e) carbon steel flanges (2).
- f) rubber gaskets (2).
- g) pyrex glass window.
- h) stainless steel trunk.
- 6) Illumination port (d-h, as above).
- 12) Flanges uniting head and body (d, e, f, h, as above).
- 19) Base plate (carbon steel).
- 20) Propeller.

Technical drawing of a mechanical part, showing a side view (top left), a top view (bottom left), and a cross-sectional view (right). The drawing includes dimensions and callouts for various features.

Dimensions:

- Overall width: 240
- Overall height: 100
- Inner hole diameter: $\phi 140$
- Outer hole diameter: $\phi 200$
- Inner hole offset: 120
- Inner hole radius: $r.95$
- Inner hole diameter: $\phi 50$
- Inner hole offset: 50
- Inner hole diameter: $\phi 12$
- Inner hole offset: 16
- Inner hole diameter: $\phi 10$
- Inner hole offset: 15
- Inner hole diameter: $\phi 12$
- Inner hole offset: 15
- Inner hole diameter: $\phi 12$
- Inner hole offset: 15
- Inner hole diameter: $\phi 12$
- Inner hole offset: 15
- Inner hole diameter: $\phi 12$
- Inner hole offset: 15

Callouts:

- 1: Top view of the part.
- 2: Side view of the part.
- 3: Cross-sectional view of the part.
- 4: Detail of the inner hole.
- 5: Detail of the inner hole.
- 6: Detail of the inner hole.
- 7: Detail of the inner hole.
- 8: Detail of the inner hole.
- 9: Detail of the inner hole.
- 10: Detail of the inner hole.
- 11: Detail of the inner hole.
- 12: Detail of the inner hole.
- 13: Detail of the inner hole.
- 14: Detail of the inner hole.
- 15: Detail of the inner hole.
- 16: Detail of the inner hole.
- 17: Detail of the inner hole.
- 18: Detail of the inner hole.
- 19: Detail of the inner hole.

Fig. 15-a

Fig. 16 - Measuring vessel for inoculum showing arrangement of electrodes.

« Fermenters of 90 and 300 l capacity for vortex and sparger aeration ».

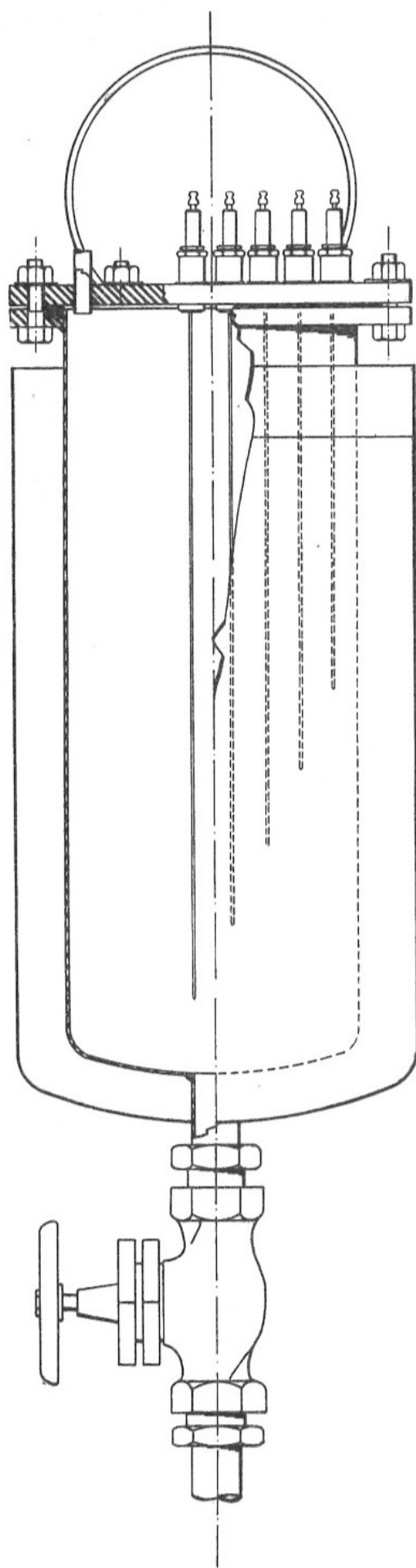


Fig. 16.

Fig. 17 - Schematic diagram of pipe and valve system for a fermentation unit consisting of seed S, intermediate S₁, and fermenter proper T.

Groups of valves and their purposes

Group	Purpose	Number in figure
A	Sampling unit.	1-4
B	Hot and cold water inlet and cold brine for jackets.	5-18
C	Steam inlet and liquid outlet for jackets.	19-28
D	Air inlet.	29-35 and 62
E	Air outlet.	40
F	Sterile addition of liquids.	36-39 and 8' 9' 10'
G	Drainage.	41-46

Explanation of symbols

AR = air inlet.	C = hooded sampling point.
AC = hot water inlet.	D = reduction valve (for air).
AF = cold water inlet.	FA = air filter.
SA = cold brine inlet and return.	H = addition vessel.
Sc = steam outlet (to collector).	I = port closed by pipe cap.
Si = security valve (to waste).	K = measuring vessel.
St = waste (to drain).	M = pressure gauge.
VA = air outlet (to flowmeter).	V.E. = electromagnetic valve.
Vc = waste (to atmosphere).	
VP = steam.	

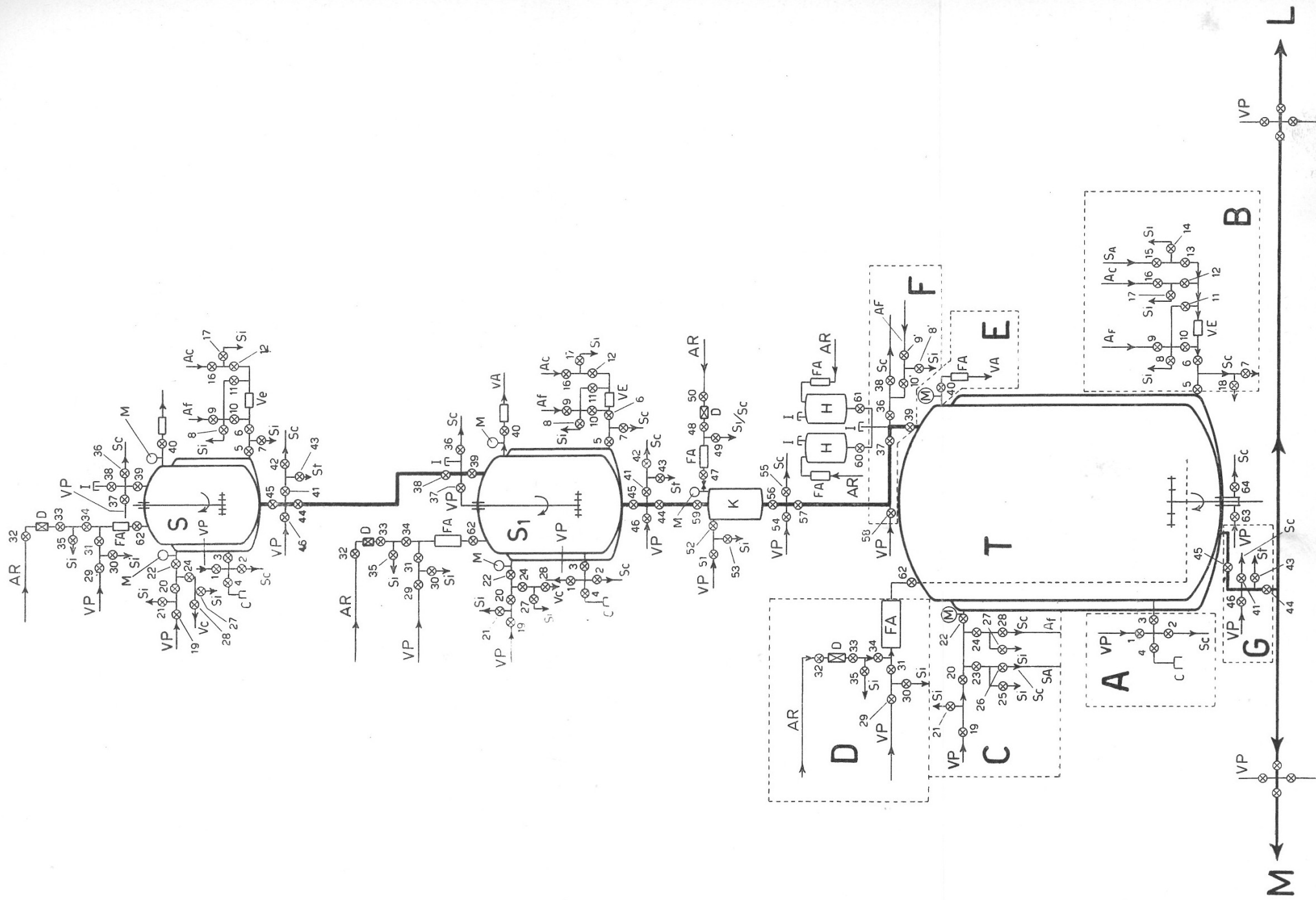


Fig. 17

«Fermenters of 90 and 300 l capacity for vortex and sparger aeration».

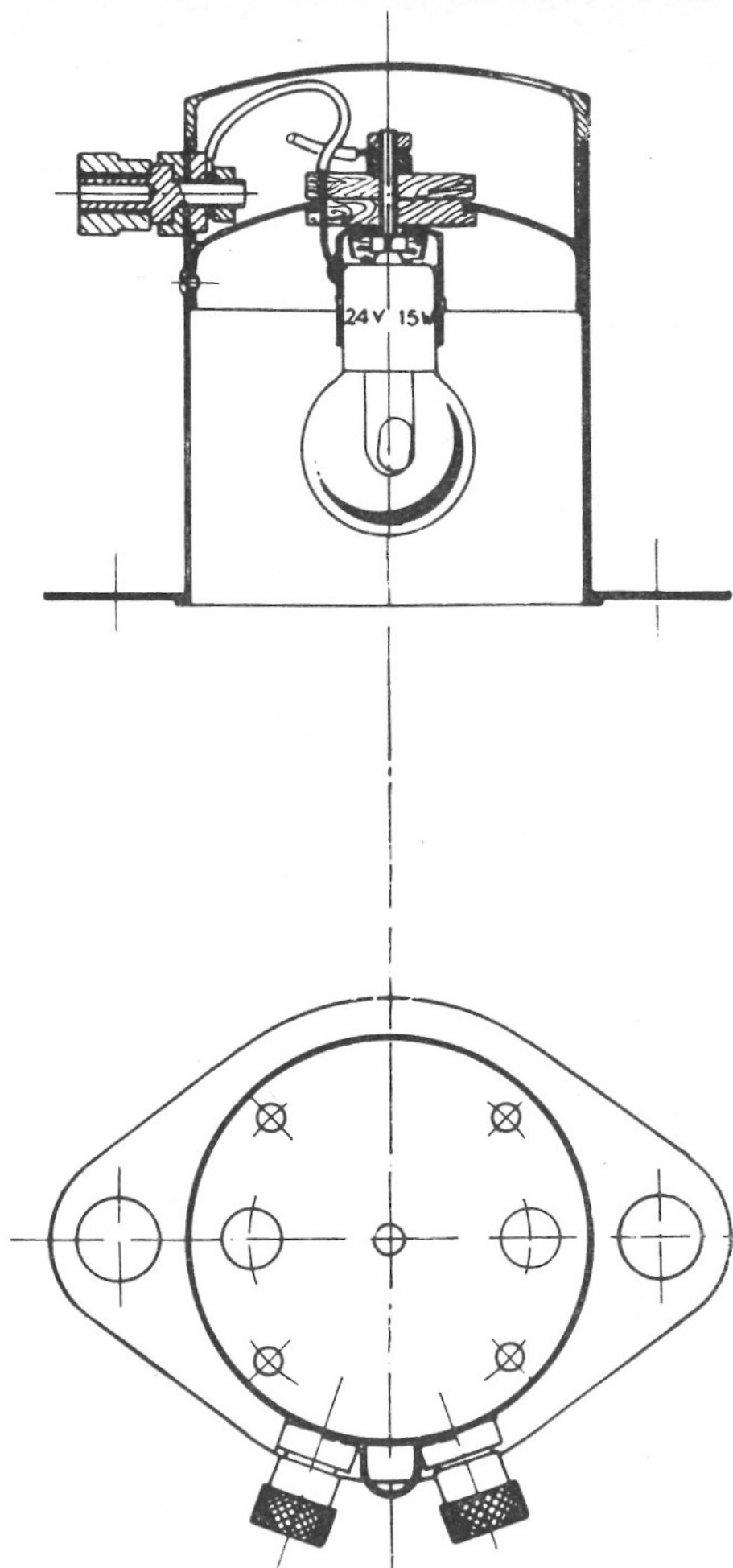


Fig. 18 - Illumination unit for seed, 90 l and 300 l fermenters.

« Fermenters of 90 and 300 l capacity for vortex and sparger aeration ».

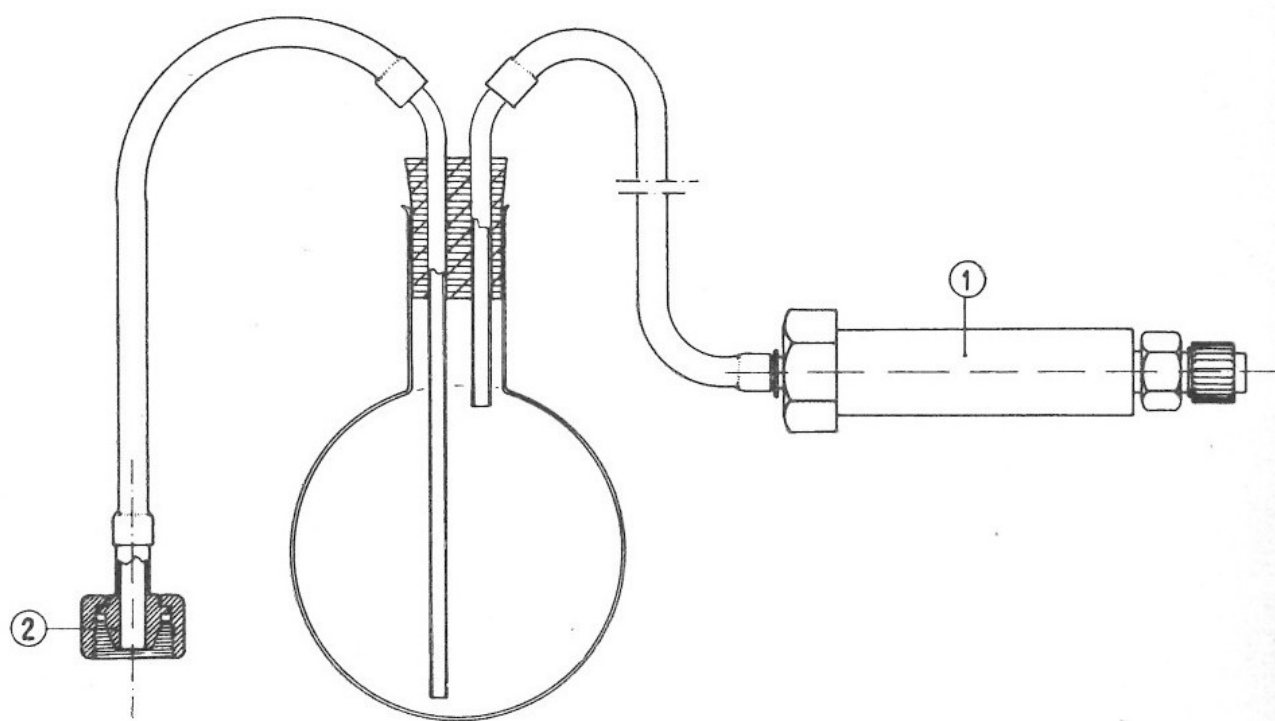


Fig. 19 - Transfer flask for inoculum.

- 1) Air inlet filter.
- 2) Half coupling.

unit of the 90 l fermenter (fig. 7 and 8). The bearing and stuffing box unit described above has given satisfactory service. However, during the course of the work a new and improved type of stuffing box has been developed by two of the authors which is described in detail elsewhere in this volume (PALADINO and UGOLINI, 1954).

The new agitation unit is located at the bottom of the tank (see fig. 11) and thus makes possible the use of a much shorter propeller shaft, which represents an important advantage particularly for high speed agitation. The driving motor can be located on the ground, instead of, as in the usual system, being attached to the fermenter head; this conveys to the fermenter a greater stability and keeps it practically free from vibration. As parts of the bearing and stuffing box unit are in direct contact with the culture fluid it is necessary to isolate it from the outside atmosphere by means of a steam seal. Details of the construction of the bearing and stuffing box unit are given in the following publication.

The new type of bearing and stuffing box unit has been substituted for the older model in the 300 l fermenters, whereas no change has been made in the 90 l fermenters. The model has given excellent service from the mechanical point of view, and has never given any trouble with regard to contamination.

Many fermentation cycles, some of a duration up to one month, have been completed with no interval for maintenance. During this time no sign of mechanical defect has become apparent in the bearing and stuffing box unit nor has it ever been the cause of a contamination.

*Filling and illumination ports, flange for sterile addition,
foam detecting electrode, thermometer wells.*

All these particulars of the fermenter head are identical with or very similar in construction to the corresponding parts of the 90 l fermenter which have been described above in detail. Constructional details are given in fig. 6, 7, 8, 8-a and 9 (Ncs 2 flange for sterile addition, 4 thermometer wells, 3 foam detecting electrode, 6 filling port, 7 illumination port).

Fermenter body.

The fermenter body (fig. 6 and 7) is a stainless steel cylinder of approximately 300 l capacity, with a dished bottom and a mild steel

flange recessed for taking a gasket (12 fig. 7, details in 12 fig. 8-a). Temperature regulation is effected by circulating water through a jacket of carbon steel, welded to the body (fig. 6 and 7).

The stainless steel plate of which the fermenter body is made has a thickness of 4 mm. and is able to withstand a working over pressure of 3 atm.

The position and number of flanges welded to the fermenter body and jacket is the same as for the 90 l fermenter (for details see fig. 6, 7, 8 and 9). The heat insulation of the jacket is also the same.

*Support of the fermenter. Tightening of fermenter head
on fermenter body.*

The fermenter is supported by three legs made of iron pipe. The legs are screwed to three stout bolts welded to the fermenter jacket.

The fermenter head is fastened on to the fermenter body by means of 24 bolts and nuts passing through the peripheral flanges. (Plate 2 and fig. 6).

Transmission.

With the agitation unit fitted on the top of the fermenter, transmission is effected as in the case of the 90 l fermenter, i.e. laterally, with the motor (1 1/3 h.p.) fixed on a pivoted bracket attached to the underside of the flange of the fermenter, (see above). Adjustment of belt tension is effected in the same way as described above.

With the new bearing and stuffing box unit fitted centrally at the bottom of the fermenter, transmission is also effected laterally, the motor in this case being fixed to the pavement of the room (Plate 2). Different speeds are obtained by fitting pulleys of appropriate size on motor and agitation unit.

ACCESSORY EQUIPMENT, SERVICES AND OPERATIONAL TECHNIQUES
COMMON TO THE 90 l AND 300 l FERMENTERS.

Addition vessels.

Sterile additions to the culture medium during fermentation may be made by the use of special « addition vessels » (fig. 12) whose construction is similar to those described for 10 l laboratory fermenters (CHAIN *et al.*, 1954c) except that overpressure from a separate airline

is necessary. Each vessel is provided with a small air filter to prevent the entry of non-sterile air. The vessels are made in a range of sizes, of uniform height but of different diameters. The internal diameter and approximate total capacities are 75 mm=1 l, 110 mm=2 l, 130 mm=3 l, 160 mm=5 l, and 230 mm=10 l.

For the addition of corrosive liquids the same arrangement as used for the inoculation of the seed fermenter is utilised (see inoculation p. 109).

When the quantity of liquid to be added is small (below 50 ml) it is convenient to use a hypodermic syringe and make the injection through a rubber diaphragm (see CHAIN *et al.*, 1954c). The same arrangement as described for the laboratory fermenters has been used for the pilot plant fermenters except that the pipe couplings are larger (1/2" gas) and the diaphragms thicker (10-12 mm).

Seed fermenter.

In some cases the 90 l fermenter was inoculated with a microbial culture grown in a seed fermenter of 10 l capacity (*). The seed fermenter, made of stainless steel, was a miniature edition of the 90 l fermenter (fig. 13).

Seven openings are cut into the dished fermenter head, two less than are present in the heads of the 90 l and 300 l fermenters, the two thermometer wells entering the fermenter body sideways through the jacket. The spacing of the holes is indicated in fig. 14 (for details of head see also fig. 15 and 15a). The bearing and stuffing box unit and the power transmission is identical with that described for the 10 l laboratory fermenter (CHAIN *et al.*, 1954c). Aeration is effected by the vortex system and no provision for fitting an air sparger on the air entry is made. The construction of the fermenter body and the jacket (made of mild steel) is indicated in figs. 13 and 14. The fermenter body has a stainless steel tube with a flange welded to the bot-

(*) During the course of routine work with the 90 l. fermenters, difficulties were experienced on occasions in growing micro-organisms in the seed fermenters. This was traced to irregularities in the temperature control owing to the small capacity of the seed fermenter jacket. In consequence, the use of the special seed fermenters for the inoculation of the 90 l. fermenter has been discontinued, and the inoculum for these fermenters is now prepared in the 10 l. laboratory fermenters in their normal water baths. After the inoculum has reached the desired age, it is transferred from the sampling tube through a rubber tubing connected to the inoculation port of the 90 l. fermenters, under pressure, by a method similar to that described in the previous paper (page 72).

The elimination of the special seed fermenters considerably simplifies the layout of the pipe and valve systems of the pilot plant.

tom for drainage and transfer of the seed culture to the 90 l fermenter (9 figs. 13 and 14). The construction of the jacket is similar to that of the 90 l and 300 l fermenters, except that the two stainless steel thermometer wells in diametrically opposed position are welded to it and the fermenter body (11 fig. 14).

Measuring vessel.

In order to be able to transfer known volumes from the 90 l fermenter to the 300 l fermenter a measuring vessel (K fig. 17) with an electric level indicator is inserted between the 90 l and 300 l fermenters. The construction of the measuring vessel is apparent from fig. 16. It consists essentially of a 10 l fermenter body covered by a stainless steel plate into which are inserted 5 electrodes at different levels. The liquid enters the measuring vessel through the stainless steel tube welded to the head of the fermenter. When the liquid level reaches the height of one of the electrodes an illuminated figure appears on an indicator board, indicating the volume in litres of the liquid which has entered the measuring vessel.

Illumination.

For illuminating the interior of the fermenter a small lamp unit is fastened to the illumination port. The unit (fig. 18) is fed through a 24 v transformer from the mains. The same pattern unit is used on all sizes of fermenters.

Temperature regulation.

Temperature regulation in all jacketed fermenters is effected by circulating cold or warm water through the jackets. The flow of water is regulated by an electromagnetic valve, commanded by a contact thermometer inserted into one of the thermometer wells welded to the fermenter heads (see above), and an electronic relay.

Services. Lay-out of pipe lines.

The fermenters are supplied with the following services:

- 1) Steam
- 2) Air
- 3) Cold water
- 4) Warm water
- 5) Cold brine

The lay-out of the pipe lines for one unit consisting of a seed fermenter, the measuring vessel, a 90 l fermenter and a 300 l fermenter, is shown in fig. 17. For distinguishing the different services the international colour code has been used. In order to ensure complete sterility during the fermentations the fermenters are isolated by means of flowing steam (steam seals) in all places where there is the possibility of contact with the outside atmosphere. Thus, steam seals are placed on the sampling unit (Group A fig. 17), the drainage (Group G fig. 17), the sterile addition nozzle (Group F fig. 17) and the stuffing box unit in the case of the bottom entry agitation unit.

The pipe conducting non-sterile water (for cleaning purposes) to the fermenter is connected to the steam line so that it is isolated from the fermenter during fermentations by flowing steam. The steam after having completed its work in the individual fermenters enters a general collecting pipe leading to a condenser whence it leaves the plant (see CHAIN *et al.*, 1954b).

During the fermentation no live steam is allowed to escape from any point of the steam pipe system into the atmosphere except for short periods before and after sampling. The steam line is connected to the air line just before the air inlet filter so that air inlet and outlet can be sterilized.

The pipe system for circulating hot or cold water for temperature regulation through the fermenter jackets is in no way connected with the fermenter proper. It therefore requires no connection with the steam line for the provision of steam seals to isolate it from the fermenters. The same applies to the cold brine line which is a closed circuit (see CHAIN *et al.*, 1954b).

Security valves.

At all points of arrival of line services to the fermenter, two valves are placed in tandem to guard against the possible failure of one valve. In addition a tee, placed between the two valves of the main line, carries a third valve on its side arm. The outlet of this valve goes to waste. (See e.g. Group D (fig. 17) valves 33, 34 and 35 in the air line, similarly 29, 30 and 31 in the steam line). The valves on the side arm of the tees, termed security valves, are normally closed when the adjacent main line valves are open and conversely are open when the line valves are closed.

This arrangement serves a dual purpose: (a) it gives an immediate indication in the event of failure of the main line valves and (b) it safe-

guards against the possible mixing of services in the pipe lines in the event of a mistaken manoeuvre on the part of the plant operator. Furthermore it provides a convenient way of removing condensate during sterilisation.

TECHNIQUE OF OPERATION.

In the following sections the technique of operation of one working unit a the fermentation pilot plant is outlined, consisting of a seed fermenter of 10 l capacity (S fig. 17), an intermediate seed fermenter of 90 l capacity (S₁ fig. 17) and a 300 l fermenter (T fig. 17).

Arrangement of valves.

The characteristic feature of the position of the valves for the different services, addition vessels etc., is their arrangement in uniform and easily accessible groups in all fermenters (fig. 17).

This greatly facilitates the operation and control of the plant and the training of the personnel. Once a person has become familiar with the arrangement and the working of valves for one fermenter he is able to operate anywhere in the plant without difficulty.

Each fermenter, (seed, intermediate or main) is equipped with seven groups of valves, as follows:

Code letter in fig. 17	Purpose	Number in fig. 17
A	sampling unit	1 - 4
B	cold water, hot water, and cold brine services for fermenter jackets	5 - 17
C	steam inlet and liquid outlet for fermenter	19 - 28
D	air inlet	29 - 35 and 60
E	air outlet	40
F	sterile addition of liquids	36 - 39 and 8', 9' 10'
G	drainage	41 - 46

Preparation of culture medium.

The culture media for the 90 l and 300 l fermenters are usually prepared in the fermenters themselves while for the 10 l seed fermenters

the culture medium is made up in a separate stainless steel container and then transferred to the fermenters. The latter procedure is particularly indicated if several 10 l seed fermenters have to be used in order to ensure homogeneity of the culture medium in all fermenters.

Before introducing solutions into the 90 l and 300 l fermenters all valves are closed with the exception of a number of security valves for the different services which are closed with the exception of a number of security valves for the different services which are opened. In the following table are listed the security valves which are opened:

Group of valves	Number of valves opened
B	8, 14, 17 and 18
C	21, 25 and 27
D	30 and 35
F	8'
G	43

Next the fermenters are half filled with warm water and the solid ingredients, finely powdered, added in aqueous suspensions through the observation ports with the stirrer rotating. It is important to avoid the introduction of large pieces of solid material because if these come in contact with a fast rotating stirrer they may cause damage to the propeller blades and shafts.

The volume of the solution is then made up with water from a hose to the desired level, taking into consideration the fact that with the technique of sterilisation described below the amount of condensate accumulating during the sterilisation is minimal.

Finally the observation port is closed and the air outlet valve 40 Group E opened.

Sterilisation of fermenters and culture media.

The sterilisation of the culture medium in the 90 l and 300 l fermenters is usually carried out at temperatures between 118° C and 125° C corresponding to 0.9-1.4 atm. overpressure of steam, while the valves outside the fermenter, the air filters and bearing and stuffing box unit (if the pressure compensated model, entering the fermenter from the bottom, is used) are sterilised at 138° C, corresponding to 2.5 atm. If

is important that no pockets remain anywhere in the pipe and valve system in which this temperature is not reached.

The temperature of 138° C is maintained in the steam seals during the fermentation.

The sterilisation of the culture medium in the fermenters is conducted in such a way that the minimum amount of condensate collects and the concentration of the ingredients of the culture medium is not appreciably altered. This is achieved by first heating the culture fluid to a temperature of 100° C by passing steam through the jacket of the fermenter before passing live steam directly into the culture medium to raise its temperature to 118-125° C. In this way the amount of condensate collecting when the temperature of 200 l of culture fluid is raised from 100° C to 125° C is about 20 l.

Before raising the temperature of the culture medium to sterilisation level steam is passed through the different groups of valves in the following sequence:

- 1) B and C (fermenter jacket)
- 2) D and E (air filter, air inlet and outlet)
- 3) F (addition of liquids in the main fermenter only)
- 4) A (sampling unit)
- 5) G (drainage)
- 6) pressure compensated stuffing box and bearing unit.

The sequence of valve manipulation is as follows:

1. *Groups B and C (Jacket)*

Valve 21 (the security valve for the steam inlet) is closed and steam is slowly admitted into the jacket by opening completely valves 20 and 22 and regulating the steam flow by valve 19, simultaneously valve 5 is opened through which the condensate from the jacket is drained via valve 18. When steam begins to appear at valve 18 this is closed and valve 7 is opened through which the steam passes into the main steam collector and condenser. While the medium warms up slowly through the passage of steam into the fermenter jacket the other groups of valves of the fermenter and accessories are sterilised as follows:

2. *Group D* (Air inlet)

Steam valve 29 is opened and condensate from the line removed through security valve 30. When live steam appears at the outlet (valve 30) valve 31 is opened and simultaneously valve 30 closed. The steam thus enters the filter and by opening slightly valve 62 the steam flow is regulated so that only a small stream passes through it into the fermenter through the air sparger. In this way the full pressure in the steam line, about 2.5 atm., and the temperature corresponding to it is maintained in all its parts outside the fermenter up to and including valve 62 while the temperature inside the fermenter may be kept at the lower level of 118-125° C required for the sterilisation of the culture medium.

3. *Group F* (Addition of liquids)

The next section to be sterilised is that concerned with the addition of solutions during the fermentation. For this purpose valves 54, 57, 58, 37, 36 and 10' are opened and the condensate which has collected in the duct for the transfer of liquids from the measuring vessel is discarded through security valve 8'. When live steam appears at 8' valve 10' is closed and by opening valve 38 the steam is passed into the general steam collector and condenser. Valve 39 is then slightly opened so that a small stream of steam passes into the fermenter; the same conditions as described under 2. are thus attained. The caps C serving for closing the connections for the addition vessels are then loosened so that a small quantity of steam emerges continuously from them.

4. *Group A* (Sampler)

Valves 1 and 4 of the sampling unit are opened and condensate ejected through valve 4. When live steam appears valve 4 is closed and by slightly opening valve 2 communication with the general steam collector and condenser is established. Valve 3 is then slightly opened so that a small stream of steam passes into the culture fluid.

5. *Group G* (Drainage)

Valve 46 is opened and condensate ejected to waste through valve 43. When live steam emerges from this valve it is closed and valve 41,

leading to the general steam collector and condenser, is slightly opened. At the same time valve 45 is slightly opened and a small stream of steam passed into the culture fluid; thus the same conditions are established as at valves 62 (air entry group D) and 39 (addition of liquids group F).

6. *Pressure compensated stuffing box and bearing unit.*

Valves 63 and 64 are opened simultaneously and condensate ejected. Valve 64 is then partially closed so that sterilisation temperature is established in this unit. After steam has been passed through the above mentioned groups of valves the sterilisation of the culture medium can then proceed. When the temperature of the culture medium has reached 100° C, i.e. when steam passes abundantly through valve 40 of the air outlet, this valve is partially closed and valve 3 of the sampling unit is further opened; thus a larger quantity of steam is passed into the culture medium. Simultaneously valve 5 of the jacket drain is completely closed and valve 19 of the steam inlet into the jacket is partially or completely closed, according to necessity. It is good practice to maintain in the jacket a steam overpressure of 0.5 atm. (read on gauge M) corresponding to 110° C while the sterilisation temperature inside the fermenter is reached and maintained by adjusting the steam pressure through valves 3 and 40.

With a little experience it is possible to maintain the sterilisation temperature inside the fermenter for periods of one hour and more with a variation of not more than $\pm 1^\circ$ C. The steam leaving through the air outlet filter may be allowed to escape into the atmosphere or conducted to the general steam collector and condenser.

The sequence of valve manipulations is the same for seed fermenter, intermediate and main fermenter, with the exception of group F. This group is connected with the cold water line only in the main fermenter; consequently valves 8', 9' and 10' are absent in the seed and intermediate fermenters.

The corresponding group F in the intermediate 50 l fermenter (S_1) is sterilised as follows: valves 41 and 46 of the seed fermenter (S) are opened and the condensate ejected through valve 43. When live steam appears at this valve it is closed and valve 42 slightly opened so as to expose the tract between valves 46 to 42 to the full steam pressure of the line. Valve 37 and 36 of the seed fermenter (S) are then opened and the condensate ejected. When this has been completed valve 36 is partially closed and valve 39 partially opened in order to expose the set

of valves concerned to the full steam pressure and to allow a small stream of steam to pass into the intermediate fermenter (S_1). Cap T is then slightly loosened to allow the escape of a small amount of steam and finally valve 44 of the seed fermenter and 38 of the 50 l fermenter are opened to sterilise the transfer duct from the seed fermenter to the intermediate fermenter.

In the seed fermenter the sequence of valve operations for the valve group 36, 37, 38 and 39 is analogous to that for the corresponding group in the intermediate fermenter.

Sterilisation of measuring vessel and transfer duct.

For the sterilisation of the measuring vessel valve 51 is opened and condensate ejected through security valve 53. When live steam emerges from this valve it is closed and steam passed into the measuring vessel, through valve 52 and its air filter, through valve 47. The pressure, read on gauge M, is regulated by valve 49 which is only slightly opened to allow the passage of a small stream of steam. Valve 56 is then opened and valve 55 slightly opened. In this way nearly the full pressure of the steam line is maintained in the measuring vessel up to and including valves 56 and 49. For the sterilisation of the transfer duct from the intermediate fermenter to the measuring vessel valves 44 and 59 are opened. For the sterilisation of the duct from the measuring vessel to the fermenter, valves 56, 55 and 37 are opened and valve 36 slightly opened.

Cooling of the culture media to working temperature after sterilisation.

After the end of the sterilisation period the temperature of the culture medium is brought to working temperature in the following manner.

a) All valves commanding the inlet of steam into the fermenter are closed, i.e. valves 45 (drainage), 3 (sampling unit), 62 (air inlet) and 40 (air outlet).

b) The steam inlet valves to the jacket 19 and 20 are closed and the security valve 21 opened. Valve 7 is then closed and any condensate in the jacket ejected through 18.

c) The steam inlet valves to the air filter 29 and 31 are closed and simultaneously security valve 30 is opened. Air inlet valve 34

is then opened after closing valve 62 and steam and condensate contained in the air filter is removed through valve 35.

During the cooling down of the filter unit to room temperature the steel filter tube, which has a considerable heat capacity, remains above the temperature of the filter pack after the release of steam pressure. Thus the packing material is dried without special precautions being necessary.

d) When all condensate from the jacket has been removed as indicated under b), cold water is gradually circulated through it. For this purpose security valves 18, 8 and 27 are closed, valves 6, 10, 24 and 28 are opened and cold water is admitted through valve 9, its flow being regulated by this valve.

e) When during the cooling process the internal pressure in the fermenter has dropped to about 0,2 atm. sterile air is admitted. This is done by closing security valve 35, opening valves 33 and 62, and regulating the air flow through valve 40.

f) When the temperature of the culture medium has reached 40-45° C the automatic temperature regulating mechanism is put in action. For this purpose valve 10 is closed and valve 11 opened. The electromagnetic valve remains open until the working temperature is reached and then begins to function. When the working temperature of the fermentation is 25° C the temperature regulation is effected by circulating cold water; if it is above 30° C warm water is circulated by opening valves 16 and 12 after closing 17; the cold water valves 9 and 11 are in this case closed.

g) Finally the air pressure in the fermenter is adjusted by the reduction valve D and the air outlet valve 40; the flow rate is measured by a rotameter placed after the air outlet filter.

Sampling.

For the withdrawal of samples from the fermenter, valve 4 is completely opened and steam passed for 10 minutes through the cap of the sampling hood which has a 2 mm hole in its centre. As this hole is very small the pressure in the whole sampling unit is not appreciably diminished during the passage of steam through the cap and sterilisation of the sampling tract is achieved. After the end of the 10 minutes sterilisation period valves 1, 2 and 4 are simultaneously closed and the sampling unit is allowed to cool to room temperature. This

is accomplished in 20-30 minutes. The cap of the sampling hood is then removed, the sampling hood flamed, valve 4 opened so that condensate may run out, the sample flask put under the hood with sterile precautions, valve 3 opened, the desired quantity of culture fluid aseptically withdrawn, valve 3 closed, the flask containing the sample removed under sterile conditions, the cap screwed on the sampling hood, valve 1 opened and the sampling outlet is washed with steam for a few minutes, valve 4 closed and valve 2 partially opened so that the steam seal is once again established.

Inoculation.

The technique of inoculation is the same for the seed as for the intermediate fermenter.

The suspension of spores or of vegetative forms of the organisms under study serving for the inoculation of seed or intermediate fermenter is contained in a round bottom flask of suitable dimensions wrapped in a towel to protect the operator against the danger of an explosion when overpressure is applied during the transfer of the contents into the fermenters (see fig. 19).

The round bottom flask is closed by a rubber bung provided with two holes through which pass two metal tubes, one reaching to the bottom of the flask and connected through a flexible rubber tubing of suitable length with a half-coupling (2 fig. 19), the other just protruding into the flask through the rubber bung and connected with a small air filter (1 fig. 19) which terminates in a rapid hose coupling. For the transfer of the inoculum to the fermenter, valves 36, 37 and 38 are closed and the whole unit allowed to cool. When a temperature of about 40° C is attained the cap covering the half-coupling of the inoculation nozzle is removed, the half-coupling flamed and connected with the half-coupling of the inoculation flask. The pressure inside the fermenter is now released to about 0.25 atm. The air filter of the inoculation vessel is then connected with the compressed air line and by opening valve 39 the contents of the flask are transferred to the fermenter at a pressure slightly above that inside the fermenter, say 0.5 atm.

When the inoculation flask is empty valves 38 and 39 are closed (in the case of the intermediate fermenter 38 remains closed during the operation) and by opening valves 37 and 36 the steam seal is established again. The air line is disconnected from the air filter of the inoculation flask which brings its pressure to atmospheric. The half-

coupling of the inoculation flask is removed from the inoculation nozzle of the fermenter and the protecting cap replaced, first loosely so that steam may pass through its thread to wash the inoculation nozzle, then, after a few minutes, tightly.

Sterile transfer of culture fluid.

a) From seed fermenter to intermediate fermenter.

The transfer duct connecting the two fermenters is cooled to room temperature by closing valves 41 and 46 of the upper steam seal and simultaneously the outlet valves 36 and 37 of the lower steam seal. Next valves 44 and 38 are opened. When the transfer duct has reached a temperature of about 35° C the pressure in the intermediate fermenter is reduced to about 0.25 atm. by adjusting air inlet valve 62 and air outlet valve 40. The pressure in the seed fermenter is then increased to about 1.25 atm. and transfer of the culture fluid from the seed to the intermediate fermenter effected by opening simultaneously valves 39 and 45. When the transfer is completed (this can be seen through the observation port in the intermediate fermenter) valves 38 and 39 are closed and the steam seal reestablished by opening valve 37 and partially, valve 36.

b) From intermediate to measuring vessel and main fermenter.

a) The steam inlet valves 46, 51, 52, 54 and 58 and the corresponding steam outlet valves 41, 47, 55, 36 and 38 are closed and the appropriate security valves 43, 53 and 49 are opened; all steam is released from the system through the latter. The whole transfer duct is allowed to cool to room temperature and the process is accelerated by passing through the transfer duct a stream of sterile air from the main fermenter. For this purpose valve 39 is opened and the air flow regulated by air outlet valve 40 so that part of the air passes through the transfer duct and the measuring vessel and leaves through the air filter at valve 49. This manipulation not only shortens the cooling period, but keeps the whole tank under positive pressure and thus reduces the risk of contamination.

b) After the transfer duct has reached room temperature the air inlet valve 39 to the main fermenter, security valve 49 and the drainage valve 56 of the measuring vessel are closed. At this stage air inlet valve 50 is opened and, valve 48 remaining closed, the air pressure is set to 2 atm. by adjusting the reduction valve D so that when, at a

later stage, the transfer of culture fluid has to be effected from the measuring vessel to the main fermenter, this pressure can be exerted on the measuring vessel.

c) by opening security valve 49 the internal pressure in the measuring vessel is reduced to 0.25 atm. while the pressure in the seed tank is increased to about 2 atm.

d) The drainage valve 45 of the intermediate fermenter is then opened and the culture fluid passed into the measuring vessel until the electric level indicator shows that the desired volume (not above 10 l) has been transferred. During this operation the pressure in the system is regulated by valve 49.

e) After the transfer has been completed drainage valve 45 and the inlet valve 52 to the measuring vessel are closed. Air inlet valves 48 and 47 are now opened and thus a pressure of 2 atm. established in the measuring vessel. In the main fermenter the pressure is reduced to 0.25 atm. by adjusting air inlet and outlet valves 62 and 40. By opening valves 56 and 39, the contents of the measuring vessel are pressed into the main fermenter. Air inlet valves 47 and 48 are then closed and security valve 49 opened.

f) If the volume of 10 l transferred is not sufficient for the inoculation of the main fermenter the operations described under a) to d) are repeated.

g) After the final transfer has been effected the appropriate steam seals are reestablished as before the transfer, i.e. at the drainage of the intermediate fermenter by opening valves 46, 41 and 42 and closing security valve 43, and in the transfer duct and measuring vessel by opening valves 51, 52, 54, 55, 58, 36 and 38 and closing security valves 53 and 49.

Sterile addition of liquids during the fermentation.

Sterile addition of solutions during the fermentation in the main fermenter is effected by means of special addition vessels, as described above. These vessels and their contents are sterilised in an autoclave, apart from the fermenter.

The fitting of the addition vessels to their respective inlets (18 fig. 1, 14 fig. 7) and the addition of solutions under sterile conditions is done in a manner similar to that described for adding the inoculum.

The caps protecting the inlets at valves 60 and 61 are loosened so

that steam passes through their threads. After 10 minutes passage valves 37 and 36 are closed, the caps removed and the exposed half-couplings of the inlets on the fermenter head connected with the half-couplings of the addition vessels under flaming. Valve 39 is then opened and a pressure of 2 atm. applied to the addition vessels via their respective air filters by connecting them with the air line, while the pressure in the fermenter is reduced to 0.25 atm. By opening valve 60 or 61 the desired solution contained in the addition vessel may be transferred to the main fermenter; the volume passing into the fermenter can be controlled by the external liquid level indicator fitted to the addition vessel.

After introducing the desired volume of liquid, valve 60 or 61 and 39 are closed, valves 36, 37 and 38 are opened and the air line is disconnected.

Termination of fermentation.

a) Closing of steam seals.

At the end of the fermentation all steam seals are closed, i.e., valves 58, 38 and 36 (steam seal for the addition of sterile liquids), 1 and 2 (steam seal of sampling unit), 63 and 64 (steam seal of the compensated stuffing box and bearing unit), 46, 44 and 41 (steam seal for drainage). Security valve 43 is opened.

b) Refrigeration of culture fluid in fermenter.

If not required immediately for working-up operations the culture fluid may be stored in the fermenter after « freezing » the enzymatic activities of the micro-organisms by cooling to $+2^{\circ}$ to $+4^{\circ}$ C with cold brine. For this purpose the contact thermometer regulating the temperature of the culture fluid is set to the desired temperature. By suitably adjusting air valves 62 and 40 a pressure of about 0.2 atm. is maintained in the fermenter. The circulation of cooling water is stopped by closing the water inlet valves 9, 11 and 6 and water outlet valve 28, and opening security valves 8 and 27. Air enters the fermenter jacket through the latter valve, replacing the water during the drainage which is effected by opening valve 18. After the draining has been completed water return valve 24 is closed. The return valves 23 and 26 of the cold brine circulating system are opened while the security valves 25 and 14 of this system and security valve 18 through which the cooling water from the fermenter jacket has been

drained are closed. Valves 15 and 13 are now opened, allowing the cold brine to enter the fermenter jacket via the electromagnetic valve until the described temperature is reached.

In order to stop the circulation of cold brine after the culture fluid has been drained from the fermenter the cold brine inlet valves 15 and 13 and return valve 26 are closed while security valve 14 is opened. A small portable pump connected with the main cold brine reservoir through a flexible rubber hose is attached to outlet valve 18 and, after opening this valve and security valve 25 the cold brine solution remaining in the fermenter jacket is pumped back to the main reservoir. The pump is then disconnected from valve 18 which is closed and the fermenter jacket and its inlet and outlet pipes are washed abundantly with water using the circuit of valves 9, 11, the electromagnetic valve VE, 6, 5, 22, 23 and 25, after closing security valve 8 keeping open VE. After the fermenter jacket has been sufficiently washed the admission of water is stopped by closing valve 9. Water inlet valves 11 and 6 are also closed and the water remaining in the jacket drained through valve 18, air entering the jacket through security valve 25.

Drainage of the culture fluid.

For drainage of the culture fluid the thermoregulating mechanism is disconnected. Drainage may be effected via drainage valves 43 and 45, valve 44 remaining closed.

Cleaning of the fermenters.

For cleaning the fermenters after the end of a fermentation the following sequence of operations is observed.

1) All inlet and outlet valves directly connected to the fermenters are closed, i.e. valves 62, 39, 3, 45 and 40.

2) The sight glass is removed from the observation port and as cleaning fluid a solution of 10% sodium carbonate in warm water is introduced.

3) All interior parts of the fermenter body and head are carefully cleaned by brushing with this solution, with the propeller periodically in motion.

4) After the cleaning operation is terminated the cleaning fluid is removed through drainage valve 45 and valves 3 and 4 of the sampling unit.

5) The fermenter is then washed by a stream of water from a hose through the observation port and through the sampling unit via valves 4 and 3.

Routine control and maintenance after each fermentation.

1) All rubber gaskets are inspected and any defective ones are replaced.

2) All valves are inspected for tightness; any which have given trouble during the fermentation are replaced.

3) The air flow from the reduction valves is checked and if blockage of the passage ways is noticed they are dismantled and the apertures cleaned.

4) All safety valves VA are inspected and their functioning checked. If necessary the housings are cleaned.

5) The proper functioning of the electromagnetic valves of the temperature regulating unit is checked by controlling that the rate of flow of water is adequate. If necessary the valves are dismantled and the conical floats closing the valves are cleaned.

6) The transmission belts are inspected for slip and wear and adjusted or replaced where necessary.

7) The thermometer wells are inspected to ascertain the presence of a sufficient amount of oil for the immersion of the thermometers.

8) The following parts are lubricated: —

a) all threaded stems of the valves with a light oil.

b) the threaded stems controlling the membrane of the reduction valve with a few drops of light oil.

c) the springs of the safety valves.

d) the bearing and stuffing box units, with a high melting grease. by means of a suitable grease gun.

Periodic Maintenance.

After 90 working days the following maintenance operations are advisable:

1) Rubber gaskets which have deteriorated are renewed.

2) All valves are dismantled, their housings cleaned and the packing renewed where necessary.

3) The gaskets of the couplings are renewed.

4) The reduction valves are dismantled, the membranes inspected and the perfect functioning of the springs ascertained.

5) The safety valves are dismantled, the seatings cleaned and the correct pressure of the springs is ascertained.

6) The electromagnetic valves are dismantled and the conical plunger and its seat cleaned. The proper functioning of the relays is checked.

7) The stuffing box unit is dismantled and inspected. (This operation must be done by skilled mechanics in the workshop).

8) The cover of the ball bearings of the electric motor is removed and the races repacked with grease.

SOURCES AND DETECTION OF CONTAMINATION.

Leaks: Before the fermenters are put into use for the first time it must be ascertained that all weldings in the interior of the fermenter, i.e. the seam of the fermenter and all the junctions with the fermenter jacket, are free from leaks. Small leaks manifest themselves during fermentation by a slowly developing contamination of a mixed culture of spore-forming and nonspore-forming bacteria. Pin hole leaks may be detected by placing a solution of fluorescein or a culture of easily recognisable « indicator » bacteria, such as *Serratia Marcescens* (*Chromobacterium prodigiosum*), into the fermenter jacket and ascertaining if the dye or the bacteria appear inside the fermenter in the culture fluid. In a well built fermenter of the type outlined above, provided with accessories and a system of pipe lines as described the occurrence of contaminations of the culture fluid is a rare event, usually traceable to a faulty operation.

Insufficient sterilisation of culture fluid.

One of the most common causes of contamination is the insufficient sterilisation of the culture medium or of solutions to be added during the fermentation, particularly if these contain suspensions of insoluble

particles such as calcium carbonate. The minimum sterilisation time permissible depends on the nature of the culture media and solutions to be sterilised. In the experience of the authors complex natural media containing calcium carbonate may be satisfactorily sterilised by keeping at 100° C for 20 minutes, followed by a period of 20 minutes at 125° C, provided the pH is between 5 and 6 and the culture fluid is continuously agitated during the sterilisation. At pH 7 and above considerably longer sterilisation times are required. For the sterilisation of antifoam oil the authors have found adequate a period of 90 minutes in the autoclave at about 125° C. The practice of dry-sterilising oils for 3 hours at 160° as suggested in the literature by various authors was found to be excessive and has been discontinued in this laboratory.

Sterility test.

To check sterility of the culture medium samples for sterility tests are taken at regular periods, beginning immediately after the medium has reached working temperature after sterilisation. Agar slopes and nutrient broth tubes are inoculated with the samples to be tested and incubated at 24° C and 37° C. The most frequently found contaminants are sporogenous gram-positive or gram-negative rods. Non-sporing bacteria are usually absent, except in the case of leaks from the fermenter jacket. Contamination of the samples taken immediately after sterilisation of the culture fluid or the addition of antifoam oil or aqueous solutions indicates incomplete sterilisation of either the culture fluid or the added solutions.

Insufficient sterilisation of antifoam oil.

Occasionally though rarely contaminations have been traced to insufficient sterilisation of the antifoam oil.

The opinion is frequently expressed that sterility of oils, in the absence of water, cannot be achieved with absolute certainty unless conditions of dry-sterilisation are applied, i.e. the oils are kept at 160° C for 2-3 hrs. In the experience of the authors these conditions are unnecessarily stringent; they found, at least for the quantities required for the pilot plant fermenters, i.e. up to 2000 ml, that autoclaving of the oils inside the addition vessels in an ordinary autoclave at 1.5 atm. pressure (corresponding to 127° C) for 90 mins was entirely sufficient to ensure sterility. Apparently the small amounts of condensate formed

in the addition vessels during autoclaving are sufficient for the destruction of micro-organisms and spores at the temperature stated.

Larger amounts of antifoam oil such as are required for fermenters of semi-industrial and industrial scale are sterilised routinely by the authors, with entirely satisfactory results, by passing live steam through the oil in the addition vessels at 2 atm. pressure.

Faulty valves.

Another common and important source of contamination is constituted by faulty valves. In globe valves the seating may become defective while in diaphragm valves the membrane of the diaphragm may deteriorate. These types of valves therefore need constant control and servicing. Cylindrical plug valves as described above are much less liable to develop mechanical defects; hence, if they are fitted the risk of contamination through defective valves is greatly reduced.

Foam formation.

A further cause of contamination is excessive foam formation. If foam is allowed to rise to such an extent that it reaches the fermenter head and escapes through the stuffing box and bearing unit (this possibility does not arise in the case where a stuffing box and bearing unit of the compensated type is fitted through the bottom of the fermenter) or the air filter, thus coming in contact with the non-sterile atmosphere, subsequent contamination of the culture fluid is almost a certainty. Excessive foam formation may occur not only during the fermentation, but also when the pressure inside the fermenter is released too suddenly. If the necessity of pressure reduction in the fermenter arises, for instance for the inoculation process or the transfer of liquid from one fermenter to another, it is therefore essential to release the pressure gradually and with care.

Another cause for contamination is a drop of pressure in the steam line so that the temperature of the steam seals, particularly of the sampling unit, cannot be maintained at the level necessary for sterilisation. During one period when owing to a repair one of the two steam generators of the fermentation pilot plant of this Institute was out of action the pressure in the steam line had dropped to just over 1 atm. from the normal working pressure of 2.5 atm. During this period a consecutive series of contamination of the fermenters was encountered.

When the steam pressure could be restored again to its normal level of 2.5 atm. the contaminations disappeared immediately and completely. When the fermenters have been idle for any length of time or after a heavy contamination with spore-forming bacteria it is good practice to sterilise them empty at 2.5 atm. steam pressure before filling them with culture medium.

Air supply.

No contamination has ever been traced to the air filters of the type as described above. In cases where the filters were suspected as the cause of contamination the authors have tested their efficiency by spraying a suspension of indicator bacteria into the air line immediately before the filter from a Collison inhaler (BOURDILLON, LIDWELL and LOVELOCK, 1948).

DISCUSSION

The above described 90 and 300 l pilot plant fermenters for submerged fermentations with non-pathogenic micro-organisms have been designed particularly for the vortex system of aeration (CHAIN *et al.*, 1952 and 1954), but, if desired, may be converted rapidly and with great ease for use of the conventional aeration method in which an air sparger and baffle plates are employed.

The literature contains several descriptions of fermenters with similar or somewhat larger (up to 1000 l) capacities, for work with non-pathogenic micro-organisms (STEFANIAK, GAILEY, BROWN and JOHNSON, 1946; GORDON, GRENFELL, KNOWLES, LEGGE, McALLISTER and WHITE, 1947; GARIBALDI and FEENEY, 1949; FORTUNE, McCORMICK, RHODEHAMEL and STEFANIAK, 1950; BLOM, PFEIFER, MOYER, TRAUFLER, CONWAY, CROCKER, FARISON and HANNIBAL, 1952; PFEIFER, VOJNOVICH and HEGER, 1952). The production of pathogenic micro-organisms poses special problems which have not been considered in this paper (for the construction of a 200 l fermenter for growing pathogenic bacteria see MALMGREN, HEDEN, SUNDSTRÖM and TÖRNQUIST, 1952). The method of aeration commonly used is to introduce the air at the bottom of the fermenter through an air sparger in the presence or absence of baffle plates and to disperse it by means of rotating propeller blades. Another method of aeration is used in the « Waldhof » system

industrially employed for the production of yeast. An adaptation of this system for a pilot plant scale fermenter has been described (SAEMAN, 1947). In this fermenter aeration is effected by making the culture liquid circulate over a draft tube inside the fermenter by means of a centrally placed rotating propeller. Air is partly sucked into the liquid from the atmosphere above by a vortex created inside the draft tube by the rotating propeller blades, partly it is injected into the culture liquid through an air sparger from a compressor. This arrangement is the nearest approach described in the literature to the vortex system developed by the authors. (See CHAIN *et al.*, 1954c, footnote on p. 62).

With regard to simplicity of design the fermenters described in the present work for use with the vortex system of aeration compare favourably with all other fermenter types described in the literature. Aeration rates up to 300 ml of oxygen per 100 ml of solution per hour can readily be attained both in the 90 and 300 l fermenters using the vortex system, and the authors believe that for fermentations in which the oxygen demand does not exceed this value this system provides the simplest and most convenient method of aeration up to the 300 l scale, and in some cases up to the 3000 l scale. (For use of vortex aeration in penicillin fermentations see CHAIN, CALLOW and SEKULIC, 1954a).

The above described fermenters have been in continuous use in this laboratory for over two years and have never given the slightest mechanical trouble. In particular, the bearing and stuffing box units, both the normal and compensated type, have given excellent service despite the heavy duty they have had to perform, with the propeller rotating at speeds between 800 and 1400 r.p.m. for many weeks, and sometimes months, without interruption.

The fitting of the addition vessels to the fermenter head and the sterile addition of solutions to the culture fluid are simple operations in which the danger of contamination has been practically eliminated. The same applies to the introduction of the inoculum into the seed fermenter and to the transfer of culture fluid from the seed fermenter to the main fermenter. Contaminations now occur very rarely and can usually be traced to a faulty operation such as incomplete sterilisation of the culture fluid.

The lay-out of the pipe and valve system was designed on conventional lines, with a few innovations. [See STEFANIAK, GAILEY, BROWN and JOHNSON (1946), who were the first to give a detailed scheme of the arrangement of pipes and valves for sterile operations in submerged fermentations with a 350 l pilot plant fermenter; also PARKER (1950) for some interesting details of aseptic technique as applied to

submerged fermentations]. One of the most important conditions for the abolition of contaminations and the smooth and reliable running of fermentations is that the fermenters can be fitted with valves which need a minimum of servicing. The valve problem has been given considerable attention by the authors, and after much experimentation the cylindrical plug valve has been found satisfactory and superior to all other types tested.

The technique of operation of the system of valves fitted to the fermenters and their accessories has been described in some detail for all phases of the fermentation process. This has been done particularly for the benefit of those who have had no previous experience in this field, as the literature does not appear to contain a detailed account of the technique of submerged fermentation with pilot plant fermenters.

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