5. E. B. CHAIN, S. PALADINO, F. UGOLINI, D. S. CALLOW — Pilot plant for fermentation in submerged culture.

Summary. — A pilot plant for the study of fermentations with non-pathogenic micro-organisms in submerged culture is described. The plant comprises a fermentation section with fermenters of 10, 90 and 300 l capacity, a preparation room for the isolation of the micro-organisms and the extraction and concentration of their metabolic products; and various accessory services. Details of the plant lay-out, equipment and sterile transfer manipulations from one fermenter to another are given. Some important organisational problems are mentioned, on the appropriate solution of which depends the smooth functioning of the pilot plant.

Riassunto. — Si descrive un impianto pilota per lo studio di fermentazioni con micro-organismi non patogeni in coltura sommersa. L'impianto comprende una sezione di fermentazione con recipienti di fermentazione della capacità di 10, 90 e 300 litri, una camera di preparazione per l'isolamento dei microorganismi e l'estrazione e concentrazione dei loro prodotti metabolici, e vari servizi accessori. Si danno particolari sulla disposizione dell'impianto, l'apparecchiatura e le manipolazioni per fare sterilmente i passaggi dall'uno all'altro recipiente di fermentazione. Si menzionano alcuni importanti problemi organizzativi, dalla cui soluzione appropriata dipende il regolare funzionamento dell'impianto pilota.

Résumé. — Les A. déscrivent une station pilote de fermentations en culture submergée pour des microorganismes non pathogènes. La station comprend une section de fermentation comportant des fermentateurs de 10, 90 et 300 litres de capacité, une chambre de préparation affectée à l'isolation des microorganismes, à l'extraction et à la concentration des produits de leur métabolisme, enfin les divers services accessoires. La disposition de l'installation, son équipement, les manipulations de transvasement en conditions stériles sont clairement détaillées. Il y est fait mention des problèmes d'organisation dont dépend le bon fonctionnement de la station.

Zusammenfassung. — Eine Versuchsanlage für das Studium von Gärungen in Tiefenkultur mit nicht-pathogenen Mikroorganismen wird beschrieben. Die Versuchsanlage besteht aus einem Gärungssaal, in dem Fermenter von 10, 90 und 300 l Inhalt untergebracht sind, einem Saal

für die Gewinnung von Mikroorganismen und die Aufarbeitung von Gärflüssigkeiten für die Darstellung der gewünschten Stoffwechselprodukt, sowie verschiedener Hilfsanlagen. Einzelheiten der Konstruktion der Anlage und der in ihr vorhanden Apparate und Maschinen werden beschrieben, und die Arbeitsmethoden für die sterile Ueberführung von Kulturflüssigkeit von einem Fermenter zum anderen werden geschildert.

Einige wichtige Organisationsprobleme werden erörtert, von deren geeigneten Lösung der erfolgreiche und reibungslose Ablauf der Arbeiten der Versuchsanlage abhängt.

Introduction

In the following a fermentation pilot plant containing fermenters from 10 to 300 l capacity is described, It is suitable for the study of fermentations with non-pathogenic micro-organisms, for the bulk production of the micro-organisms themselves and the extraction and concentration of their metabolic products.

In the literature several descriptions of fermentation pilot plant equipment have been reported, among which the most detailed ones are those of Jacobs, Wright and Hildebrandt (1948), Fortune, McCormick, Rhodehamel and Stefaniak (1950) and Pfeifer, Vojnovich and Heger (1952). However, though these papers contain much information of great value to the specialist on important aspects of the technique of fermentation on pilot plant scale and the working up of the desired products, they are not sufficiently detailed to enable someone without considerable practical experience in the field to project and construct a workable fermentation pilot plant. In view of this fact and the growing importance for all branches of biochemistry of methods for the large-scale production of microbial metabolites, the present authors felt that a useful purpose would be served by a description of the fermentation pilot plant, which they have developed and which has been in continuous use for over three years, in sufficient detail to enable anyone interested to copy it without difficulty. A discussion of some of the organisational problems encountered in running the pilot plant, on the appropriate solution of which its success ultimately depends, was also considered pertinent.

General Lay-out.

The general lay-out of the pilot plant is illustrated in fig. 1. It consists of a fermentation section (A fig. 1 and plates 1 and 2), a preparation room (F fig. 1 and plates 3 and 3-a) and various accessory services, such as a sterilisation room for the laboratory fermenters (M fig. 1), a solvent recovery plant (G fig. 1 and plate 4), a compressor room (B fig. 1) and a boiler room (I fig. 1).

Fermentation section.

Number and size of fermenters.

One of the most important factors limiting the number of fermenters wich can be usefully accommodated in a pilot plant is the number of service personnel available (see below). The present plant contains fortysix laboratory 10 l fermenters, eight 90 l fermenters (Plate 5) and three 300 l fermenters (Plate 6) whose construction has been described in detail (Chain, Paladino, Ugolini Callow and Van der Sluis, 1954; Paladino, Ugolini and Chain, (1954)

10 l fermenters.

The need in a fermentation pilot plant for a large number of small size fermenters is selfevident; they serve for series experiments to study the course of fermentations under different conditions.

90 and 300 l fermenters.

The availability of larger size fermenters is essential not only for preparative purposes, but also for reasons of biological and biochemical nature. For instance, if there is an effect of mechanical agitation on the morphological form and biochemical behaviour of micro-organisms, as is the case, for example, with *Penicillium chrysogenum* (Dion, Carilli, Sermonti and Chain, 1954), this effect becomes less marked as the volume of the culture fluid increases, particularly in non-baffled aeration systems; the reason for this is that per time unit the micro-organisms are less frequently subjected to the mechanical

action of the rotating propeller blades. It is therefore sometimes impossible to reproduce in small fermenters conditions existing in fermenters of larger dimensions.

Furthermore, it is difficult if not impossible to fit automatic control equipment for the addition of solutions, e.g. for pH control, to small fermenters, because of space limitations on the fermenter head; the smallest fermenter unit to which automatic equipment can be conveniently fitted should have a capacity of not less than 50 l. If the pH has to be maintained constant in a large number of fermenters manual adjustment is often out of the question because of labour and time involved and automatic pH measurement and control is the only satisfactory method.

3000 l fermenter.

In addition to the 90 and 300 l fermenters, the authors have available in another building a fermenter with a total volume of about 3000 l which they have found a very useful addition to their pilot plant; this fermenter was found particularly important when studying scaling-up effects from the laboratory to the industrial scale.

Arrangement of fermenters.

The disposition of the fermenters is shown in fig. 2. The small fermenters are housed partly in the main fermentation room A (fig. 1 and Plate 1), partly in the corridor Q where suitable wall space was available (Plate 7).

The 90 and 300 l fermenters are all housed in the main fermentation room. The pipe lines are arranged in such a way that transfers can be made from any one fermenter to any other (fig. 3).

For each set of two 90 l fermenters one 10 l seed fermenter is provided (*) (Plate 8), while the three 300 l fermenters are inoculated from a 50 l seed fermenter, which in turn may be inoculated either directly with a spore suspension or through a 10 l seed fermenter with a suspension of vegetative micro-organisms (fig. 2 and 3).

(*) See Paladino, Ugolini and Chain 1954, foot note on page 99.

Sterilisers.

The culture medium may be sterilised in the fermenters themselves or if required, in separate cookers, (13 fig. 2 and Plate 9) two of which are provided for the purpose. The availability of special cookers is useful for several purposes, for instance for replacing culture medium in the fermenters during the course of a fermentation without interruption, and for the separate sterilisation of solutions which undergo changes when heated together with other constituents of the culture medium. The cookers are Pfaudler jacketed, glass lined reaction kettles each with a total capacity of 200 l. They are adapted for their service as sterilisers as illustrated in Plate 9 and are connected with the same services, apart from hot water, as are provided for the fermenters of 90 and 300 l capacity, (PALADINO et al., 1954) with a similar arrangement of valves.

Agitation of the culture fluid during sterilisation is important particularly if solid material is present.

In the present sterilisers agitation is, for the time being, effected by bubbling a stream of air through the culture fluid, but provision is made for installing the much more effective mechanical agitation. The flanges welded centrally to the kettle heads for the purpose of carrying stuffing box and bearing units for propeller shafts are at the moment closed by cover plates.

The sterilisation of the culture fluid in the steriliser is done in the same way as has been described for the 90 and 300 l fermenters (PALADINO et al., 1954).

Valve and pipe system.

The arrangement of valves and services for a typical individual fermenter unit has been described in detail in the preceding paper (PALADINO et al., 1954, fig. 17). It has been reproduced in detail in fig. 3-a. Each fermenter is provided with an exact replica of this arrangement. All valves are arranged in easily accessible groups.

The general lay-out of the pipe system connecting the different fermenters is shown in fig. 3.

All pipes through which steam or culture fluid are passing are made of stainless steel. In the first version of the pilot plant it was considered iron steam pipes would meet the requirements, but expe-

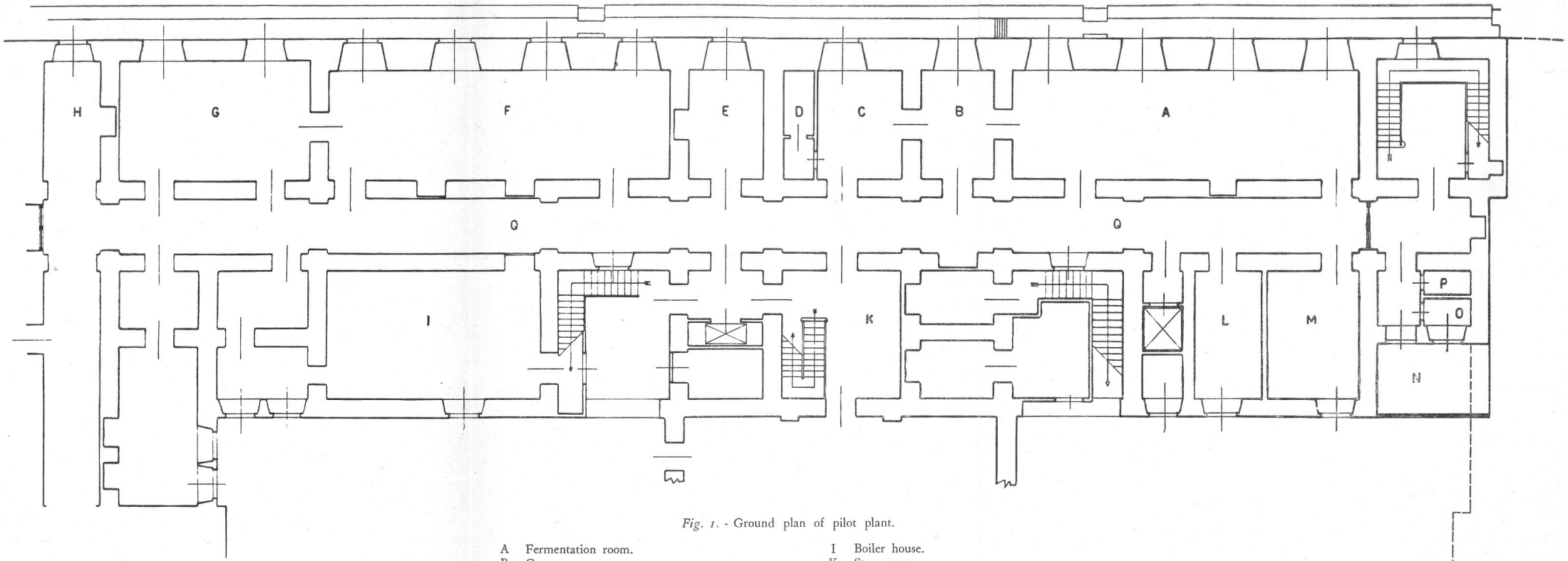

Fig. 1. - Ground plan of pilot plant.

A Fermentation room.
B Compressor room.
C Small works laboratory for routine controls.
D Cold room.
E Incubator room.
F Preparation room.
G Solvent recovery plant.
H Plant workshop.
I Boiler house.
K Store space.
L Office
M Room for sterilisation of small fermenters, media making and glass washing.
N Store space.
O Lavatory.
P Showers.
Q Corridor.

Fig. 2. - Fermentation room.

1) Compressors for refrigeration.
2) Cold brine reservoir.
3) Air compressors.
4) Air reservoirs.
5) Air and gas distributing lines.
6) Cold water distributing line.
7) Water softener.
8) Electric steam generator.
9) Unit of 10 l laboratory fermenters.
10) 300 l fermenters.
10-*a*) 5 l seed fermenters for 10.
10-*b*) 50 l seed fermenters.
10-*c*) Measuring vessel.
11) 200 l fermenter.
12) 90 l fermenters.
12-*a*) 10 l seed fermenters for 12.
13) Sterilisers, 200 l capacity.
14) Vertical autoclave, 200 l capacity.
15) Electrical distributor and switch board.
15-*a*) Electrical indicator panel.
16) Drainage channels. The arrows indicate connection with main drain.
17) Entry to subterranean gallery, containing main drainage channels.

Pilot plant for fermentation in submerged culture.

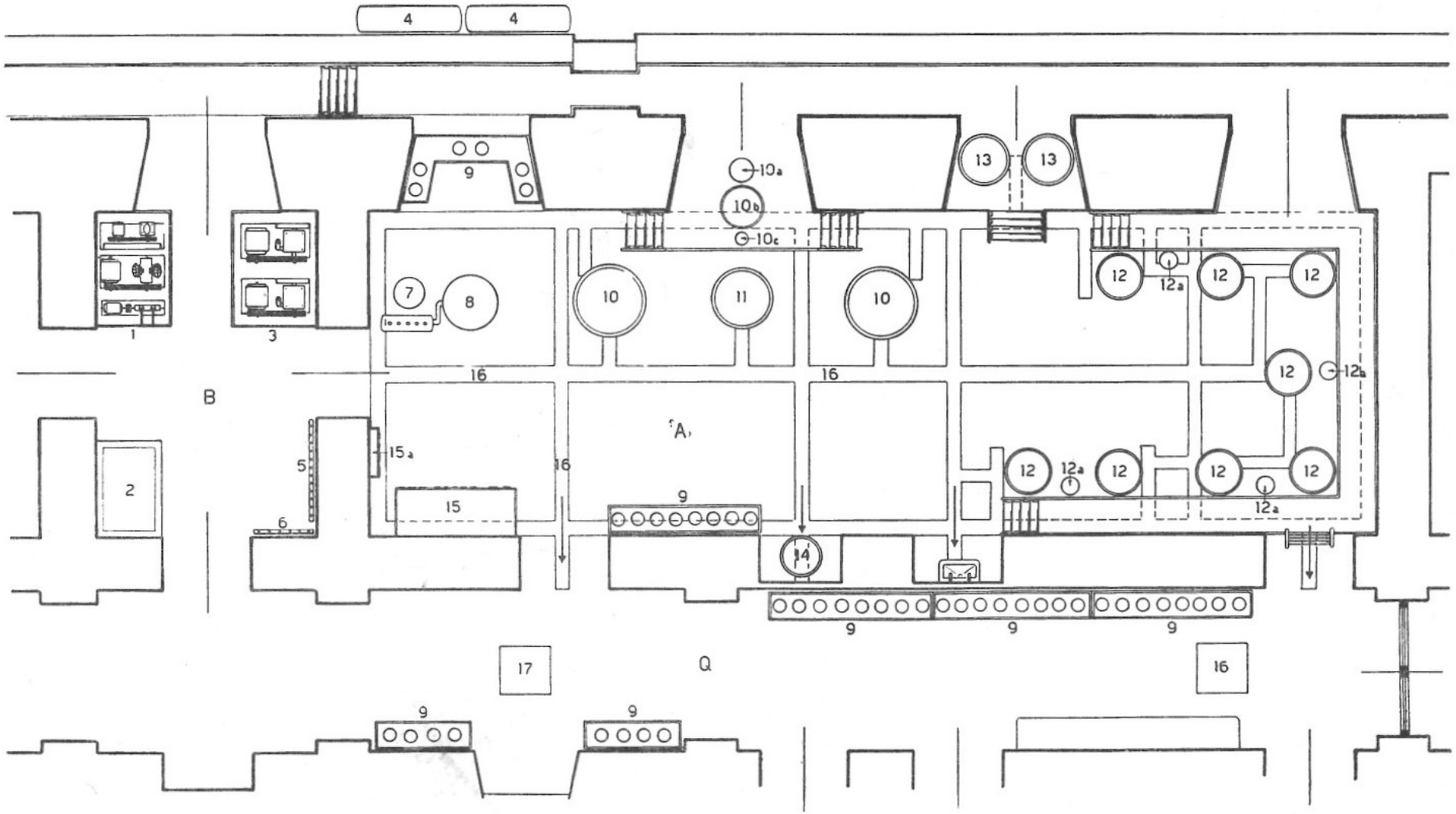

Fig. 2.

Fig. 3. - Schematic diagram of pipe system connecting the different fermenters in the fermentation room.

A-G = Valve groups. For details see Fig. 3-a.
L-M = Pipe line connecting 90 l and 300 l fermenters.
S, S_2, S_3, S_4 and S_5 = 10 l seed fermenters.
S_1 = 50 l intermediate fermenters.
ST_1 and ST_2 = Sterilisers (mash cookers).
T_1, T_2 and T_3 = 300 l fermenters.
T_4-T_{11} = 90 l fermenters.

AR = Air inlet.
AC = Hot water inlet.
AF = Cold water inlet.
SA = Cold brine inlet and return.
SC = Steam outlet (to collector).
VA = Air outlet (to flowmeter).
VP = Steam inlet.
UC = Cold water outlet.

C = Hooded sampling point.
K = Measuring vessel.
I = Port closed by pipe cap.
M = Pressure gauge.

Pilot plant for fermentation in submerged culture.

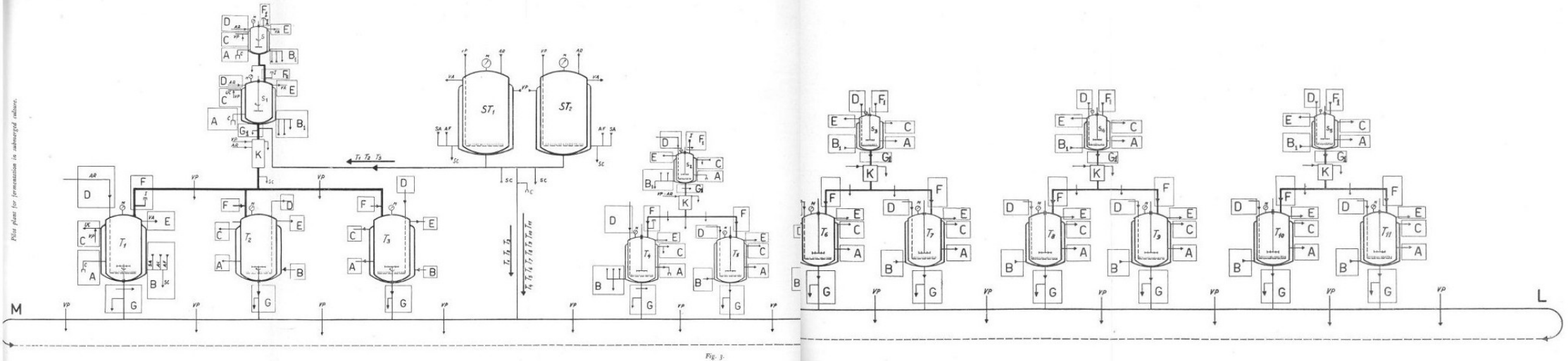

Fig. 3.

Fig. 3-a. - Details of valve groups.

Group A Sampling unit.
» B Hot and cold water inlets and cold brine for jackets.
» B_1 Modification of Grup B used on seed fermenters where cold brine is not required.
» B_2 Modification of Group B used on sterilisers where hot water is not required.
» C Steam inlet and liquid outlet for jackets.
» D Air inlet.
» E Air outlet.
» F Sterile addition of liquids to fermenters.
» F_1 Sterile addition of liquids to seed fermenters.
» F_2 Sterile addition of liquids to intermediate fermenters.
» G Drainage.
» G_1 Modification of Group G used on seed fermenters.

AR = Air inlet.
AC = Hot water inlet.
AF = Cold water inlet.
SA = Cold brine inlet and return.
SC = Steam outlet (to collector).
Si = Security valve (to waste).
St = Waste (to drain).
VA = Air outlet (to flowmeter).
VP = Steam inlet.

C = Hooded sampling point.
FA = Air filter.
I = Port closed by pipe cap.
K = Measuring vessel.
M = Pressure gauge.
R = Reduction valve (for air).
VE = Electromagnetic valve.

Pilot plant for fermentation in submerged culture.

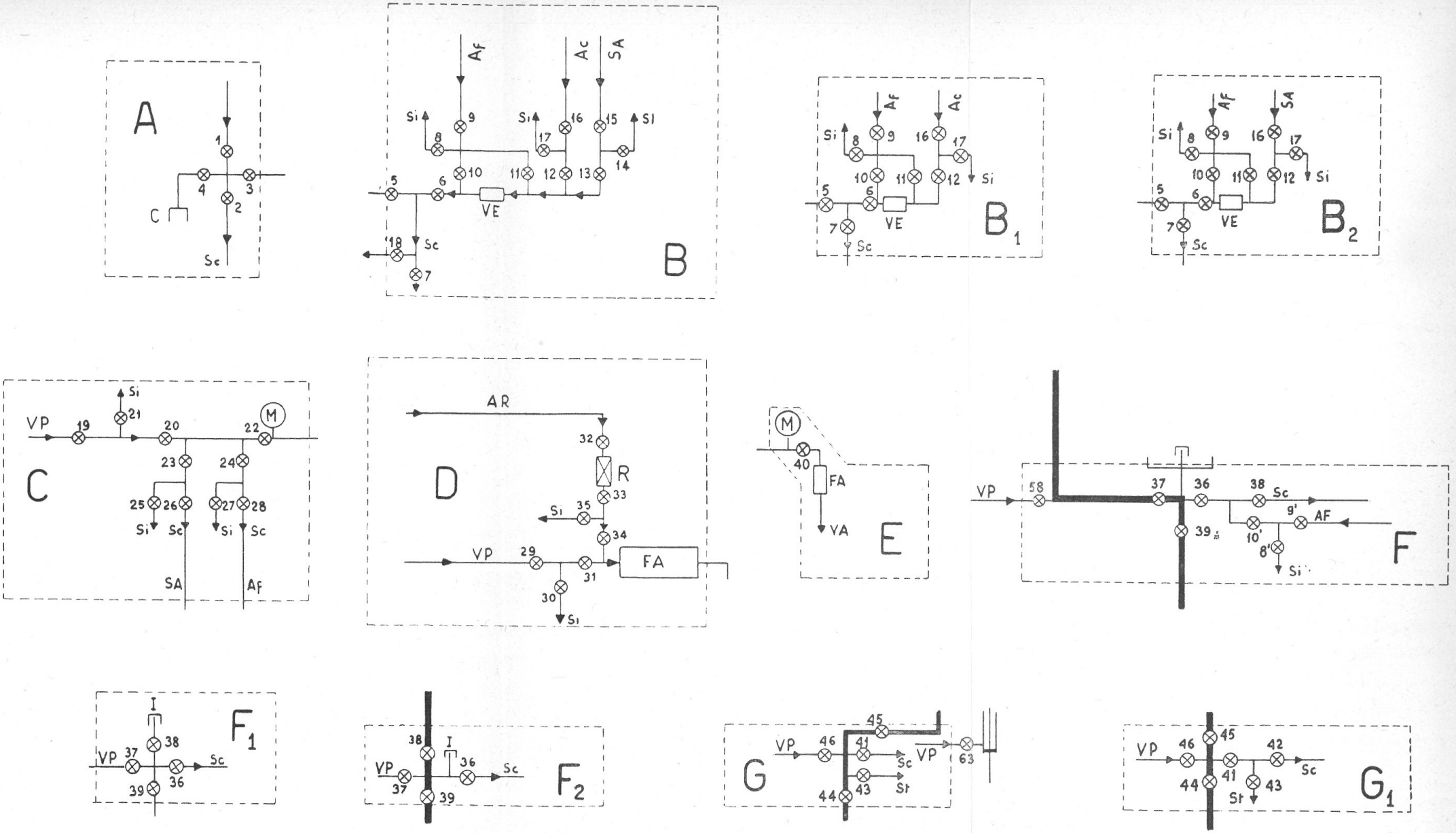

Fig. 3-a

Fig. 3-b. - Schematic diagram of pipe system used for transfer operations.

A-G = Valve groups. For details see Fig. 3-*a*.
L-M = Pipe line connecting 90 l and 300 l fermenters.
S_1 = 50 l intermediate fermenter.
ST_1 and ST_2 = Sterilisers (mash cookers).
T_1, T_2 and T_3 = 300 l fermenters.
T_4-T_{11} = 90 l fermenters.
V_1-V_4 = Volumetric calibration marks inside fermenters.
I-IX = Steam seals isolating fermenters one from another and dividing the transfer line LM into easily sterilisable short tracts.

AR = Air inlet.
AC = Hot water inlet.
AF = Cold water inlet.
SA = Cold brine inlet and return.
SC = Steam outlet (to collector).
Si = Security valve (to waste).
St = Waste (to drain).
VA = Air outlet (to flowmeter).
VP = Steam inlet.
C = Hooded sampling point.
FA = Air filter.
I = Port closed by pipe cap.
K = Measuring vessel.
M = Pressure gauge.
R = Reduction valve (for air).
T = Thermometer.

Pilot plant for fermentation in submerged culture.

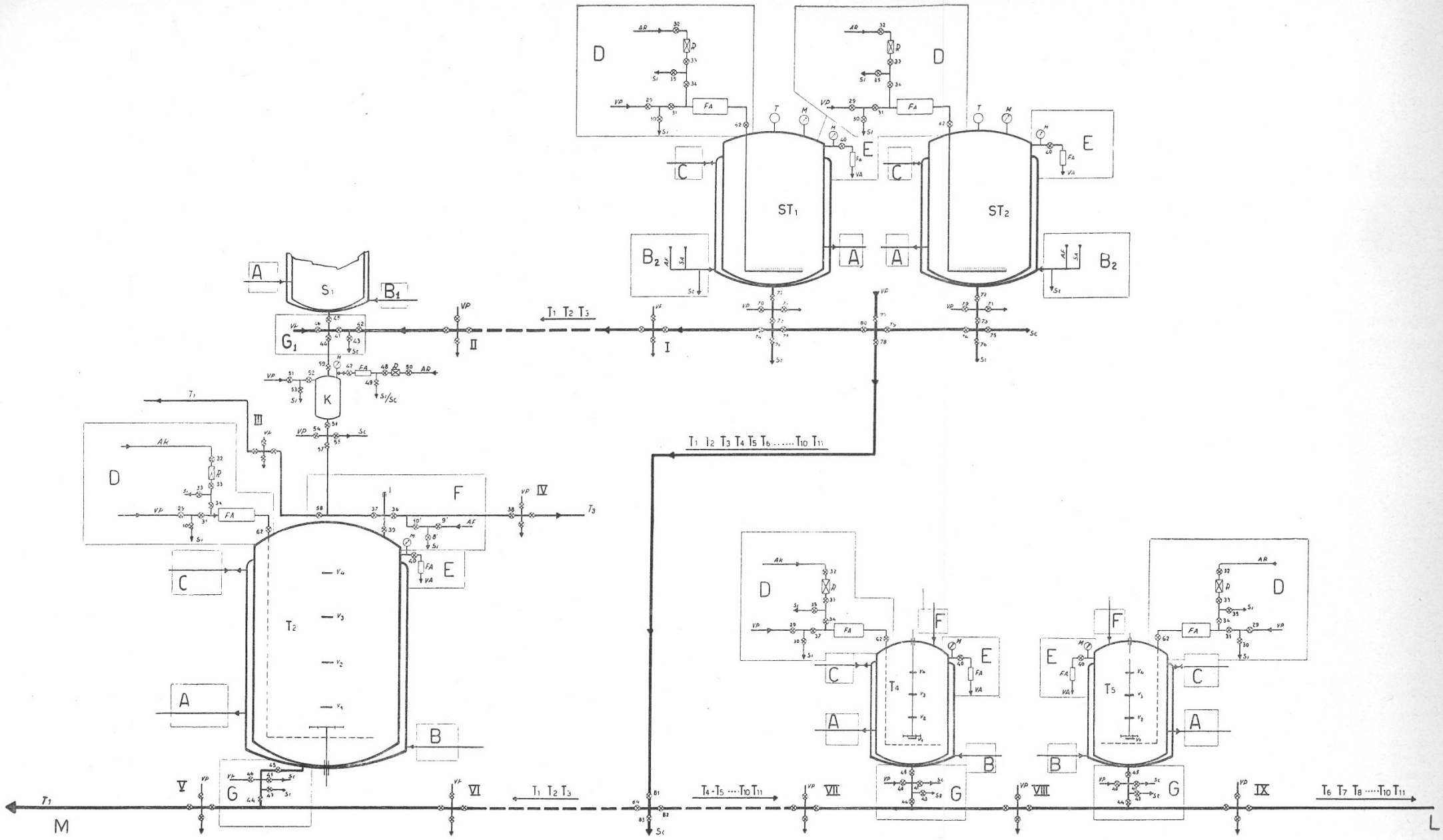

Fig. 3-b

Fig. 4. - Steam circuit.

1) Steam generator, naphtha burning, producing 100 kilos/hour of sat. steam at 4 Atm.
2) Steam generator, electric, power consumption 120 KW, producing 150 kilos/hour of sat. steam at 4 Atm.
3) Main steam supply line leading to fermentation room (pressure at arrival 3 Atm.).
4) Steam distributor line to fermenters and other equipment.
5-10) Steam line deviation for:
   5) upper steam seals for transfer from seed vessels;
   6) air filter;
   7) fermenter jacket;
   8) sample unit;
   9) steam seals for compensated stuffing boxes;
   10) lower steam seals for fermenter drain.
11) Return steam line.
12) Steam condenser. (Heat exchanger).
13) Condensate drain.
14) Entry of decalcified fresh cold water into heat exchanger.
15) Warm water line to boiler and plant.

Pilot plant for fermentation in submerged culture.

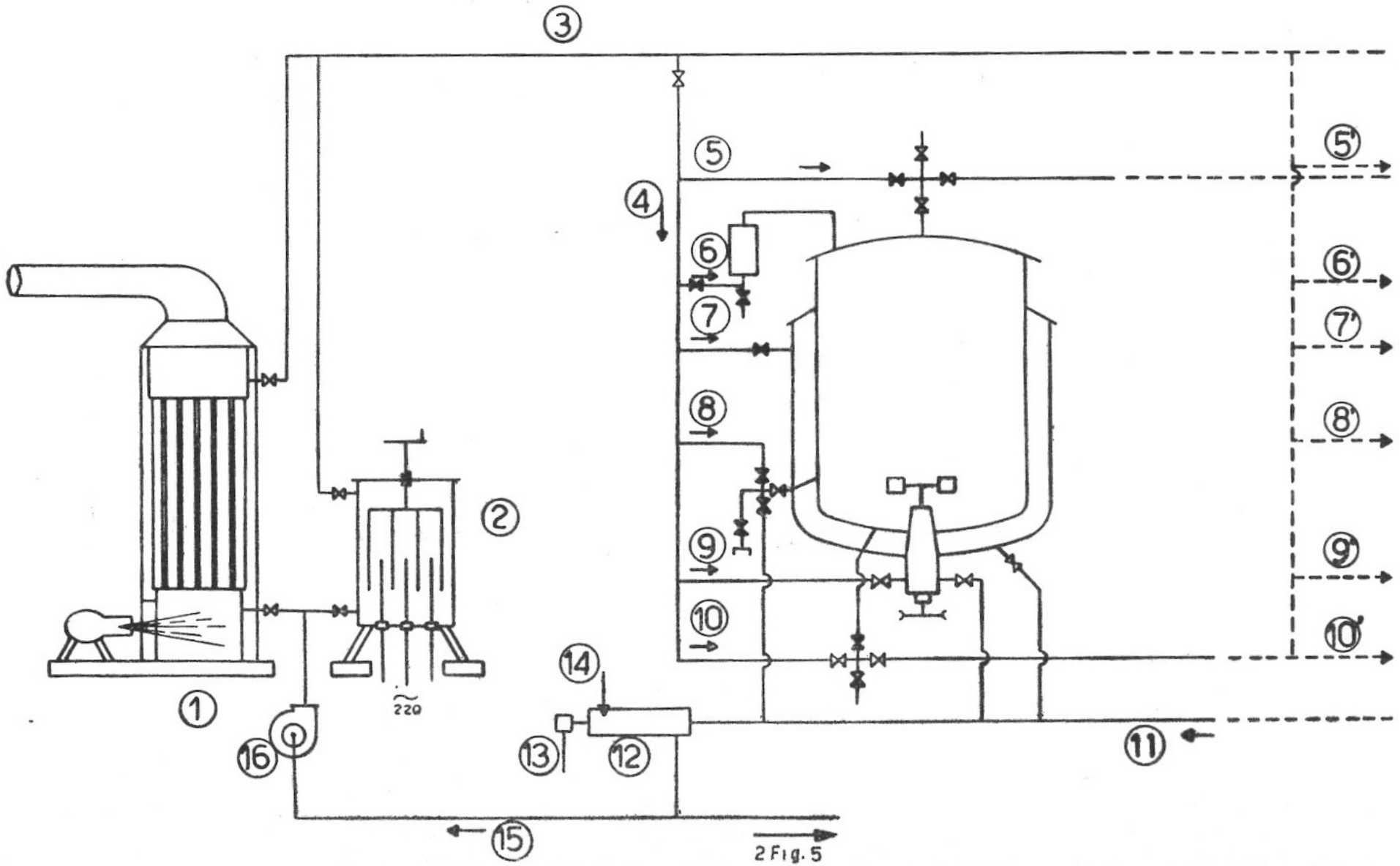

Fig. 4.

Fig. 5. - Water circuit.

1) Main cold water supply line.
2) Main hot water supply line.
3) Entry of cold water to fermenter jacket through electromagnetic valve.
4) Entry of hot water to fermenter jacket through electromagnetic valve.
5) Entry of cold water directly into fermenter jacket.
6) Entry of cold water into fermenter.
7) Electromagnetic valve.
8) Fermenter.
9) Water outlet from jacket.
10) Pipe line to main drainage.

Pilot plant for fermentation in submerged culture.

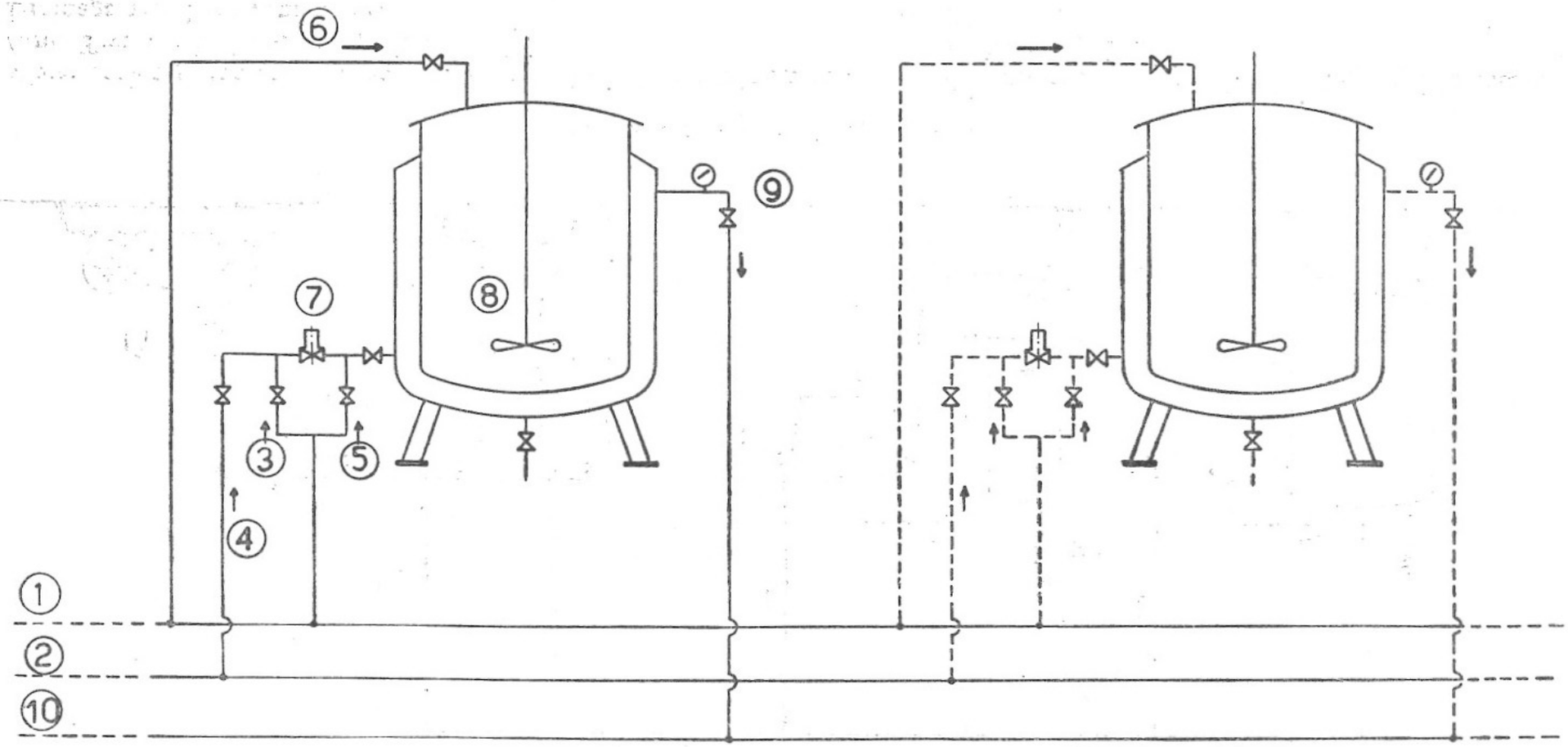

Fig. 5.

Pilot plant for fermentation in submerged culture.

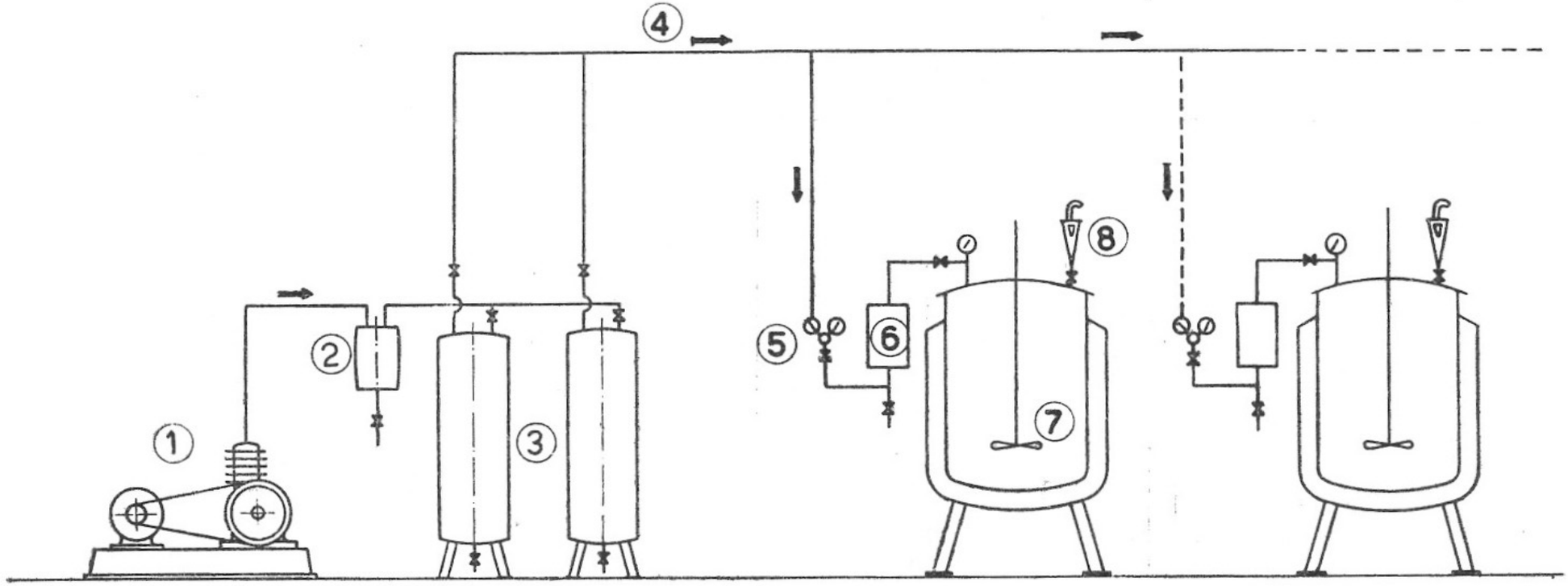

Fig. 6. - Compressed air line.

1) Water cooled piston compressor, capacity 1000 l of air per minute at 3 Atm. Two available, working alternatively.
2) Drainage vessel for condensate.
3) Reservoirs for compressed air, capacity 300 l each.
4) Compressed air pipe line leading to fermenters.
5) Reduction valve.
6) Air filter.
7) Fermenter.
8) Flowmeter.

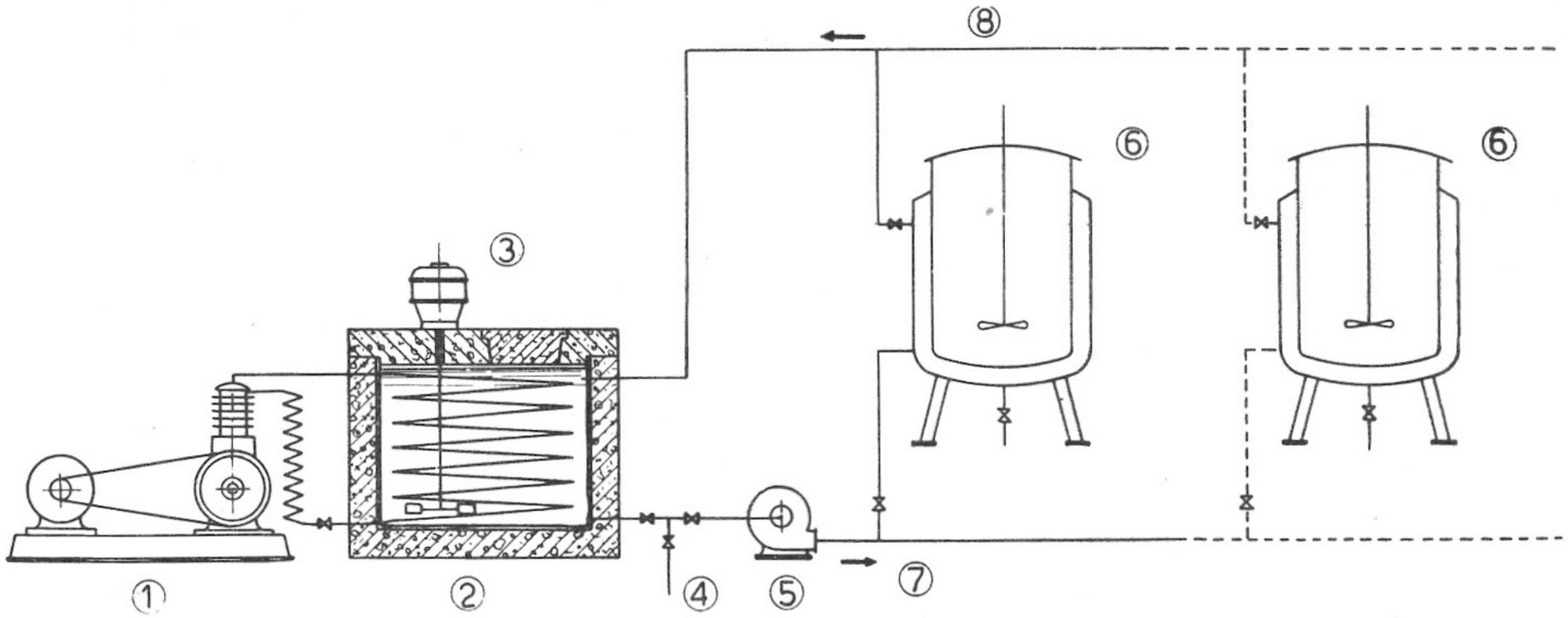

Fig. 7. - Cold brine circulation.

1) Compressor, cooling capacity 10.000 Kcals. per hour.
2) Cold brine reservoir, capacity 1000 l.
3) Agitator for cold brine.
4) Drainage of cold brine reservoir.
5) Pump feeding cold brine to the fermenters.
6) Fermenters.
7) Pipe line carrying cold brine to fermenters.
8) Pipe line returning cold brine to reservoir.

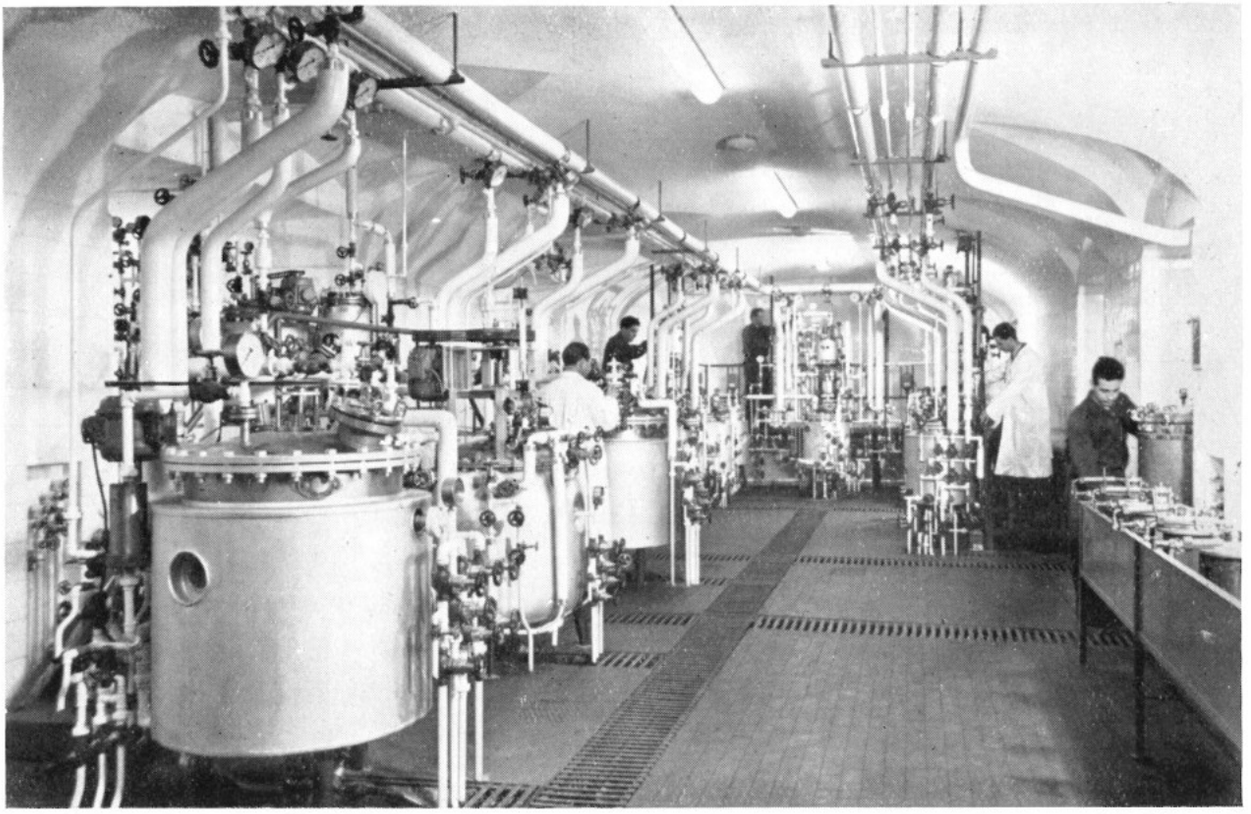

Plate 1. - Fermentation room (A fig. 1), showing left: 300 l fermenters, centre: 90 l fermenters, right: bath with 10 l fermenters.

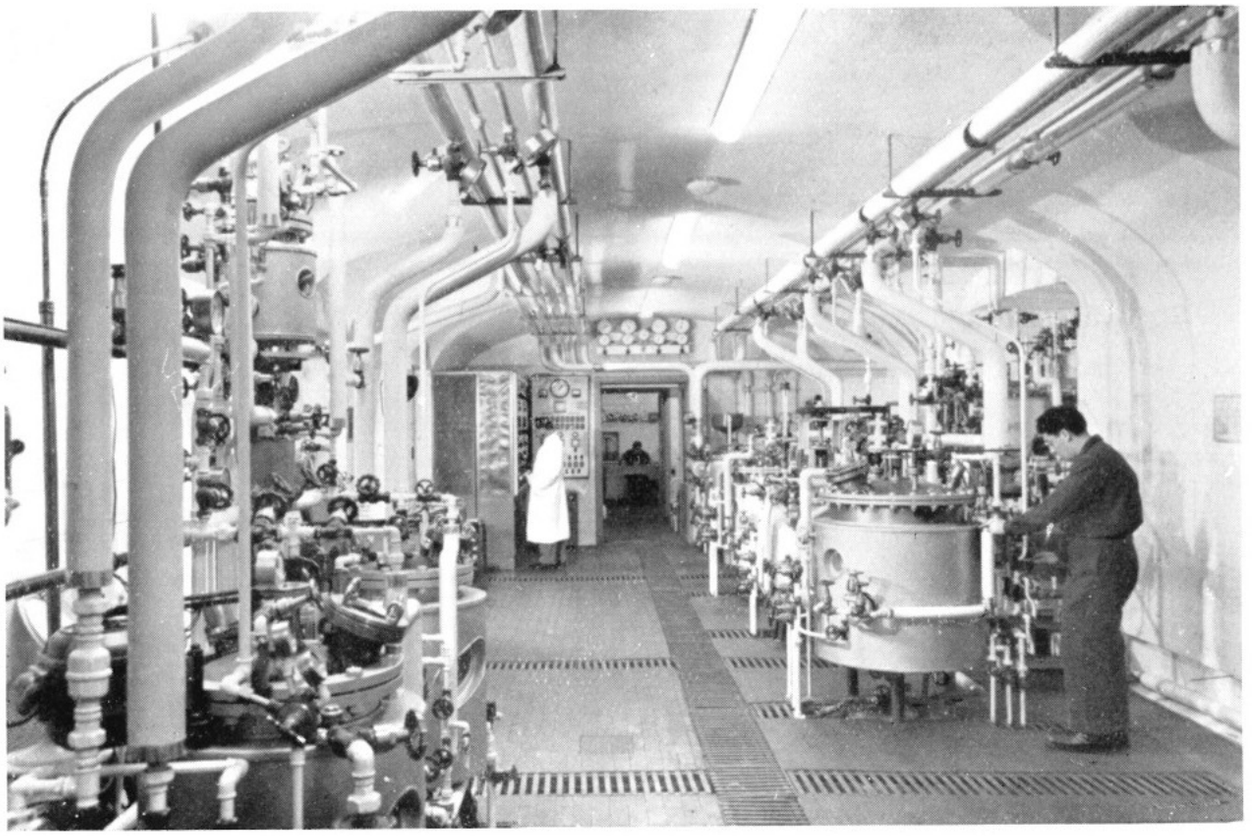

Plate 2. - Fermentation room (A fig. 1), showing left: 90 l fermenters, centre: electrical control panel and entrance to laboratory for routine controls (C fig. 1), right: 300 l fermenters.

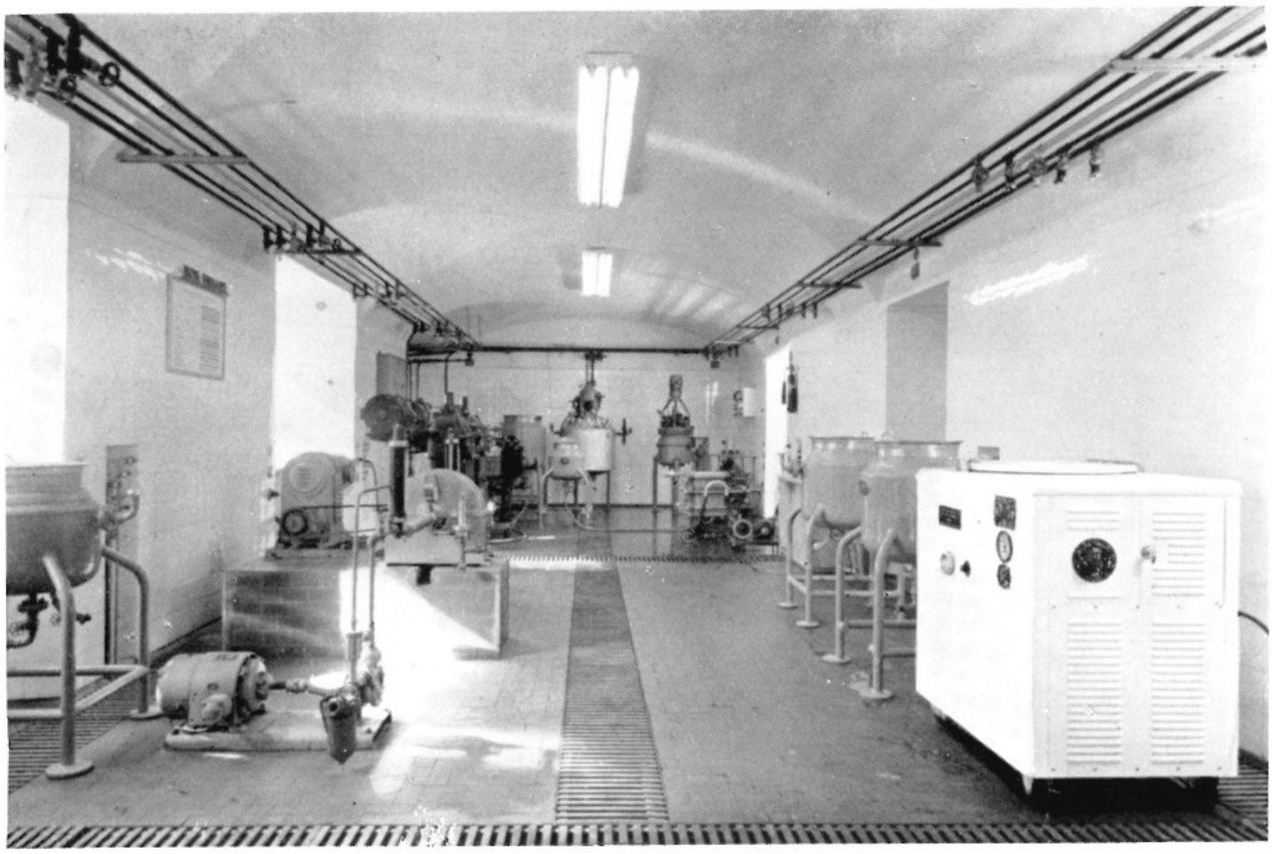

Plate 3. - Preparation room (F fig. 1), general view.

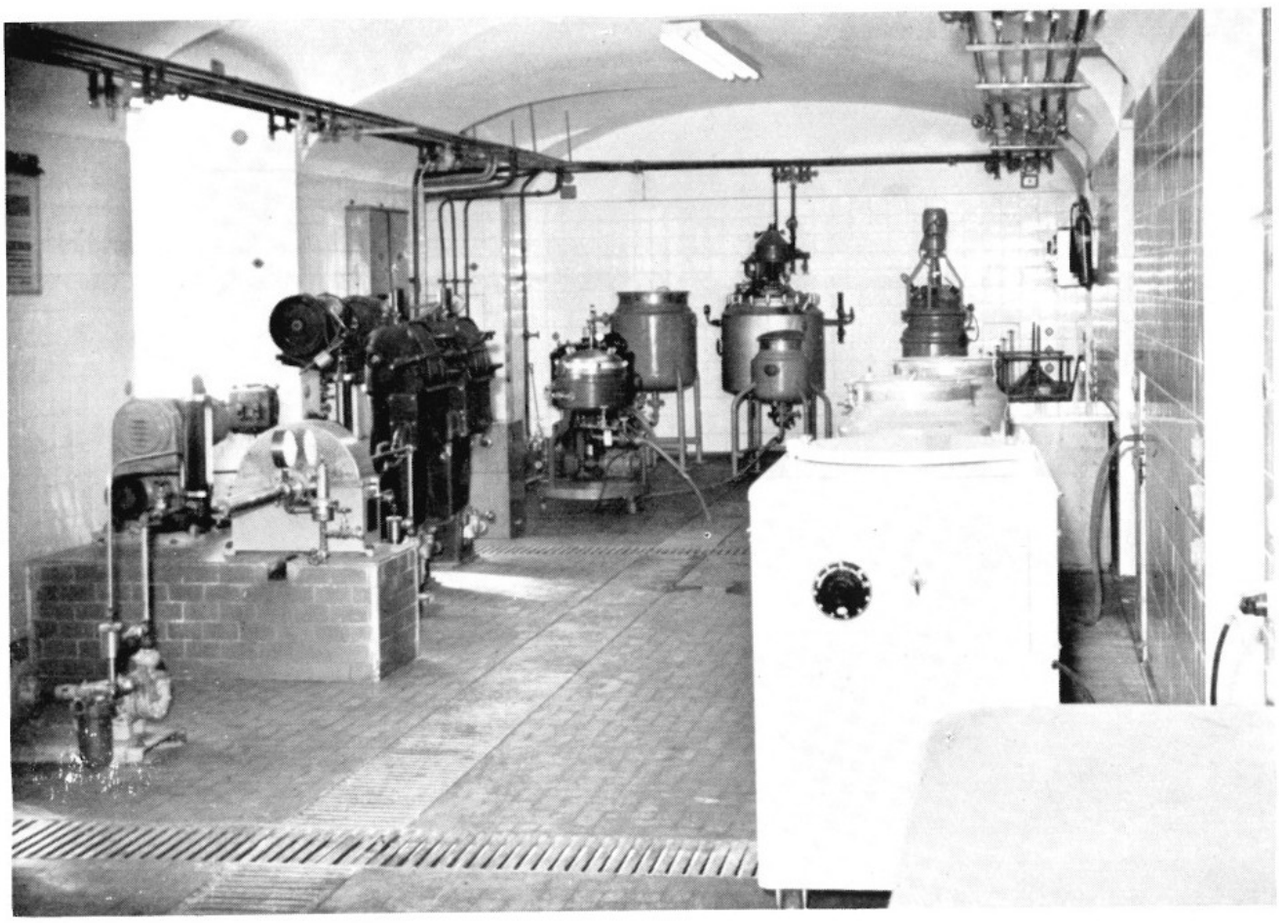

Plate 3a. - Preparation room (details of fig. 3), left: Podbielniak extractor, Sharples centrifuges, Sparkler filter, centre: jacketed storage vessels and reactors, right: jacketed storage vessels, refrigerated centrifuge.

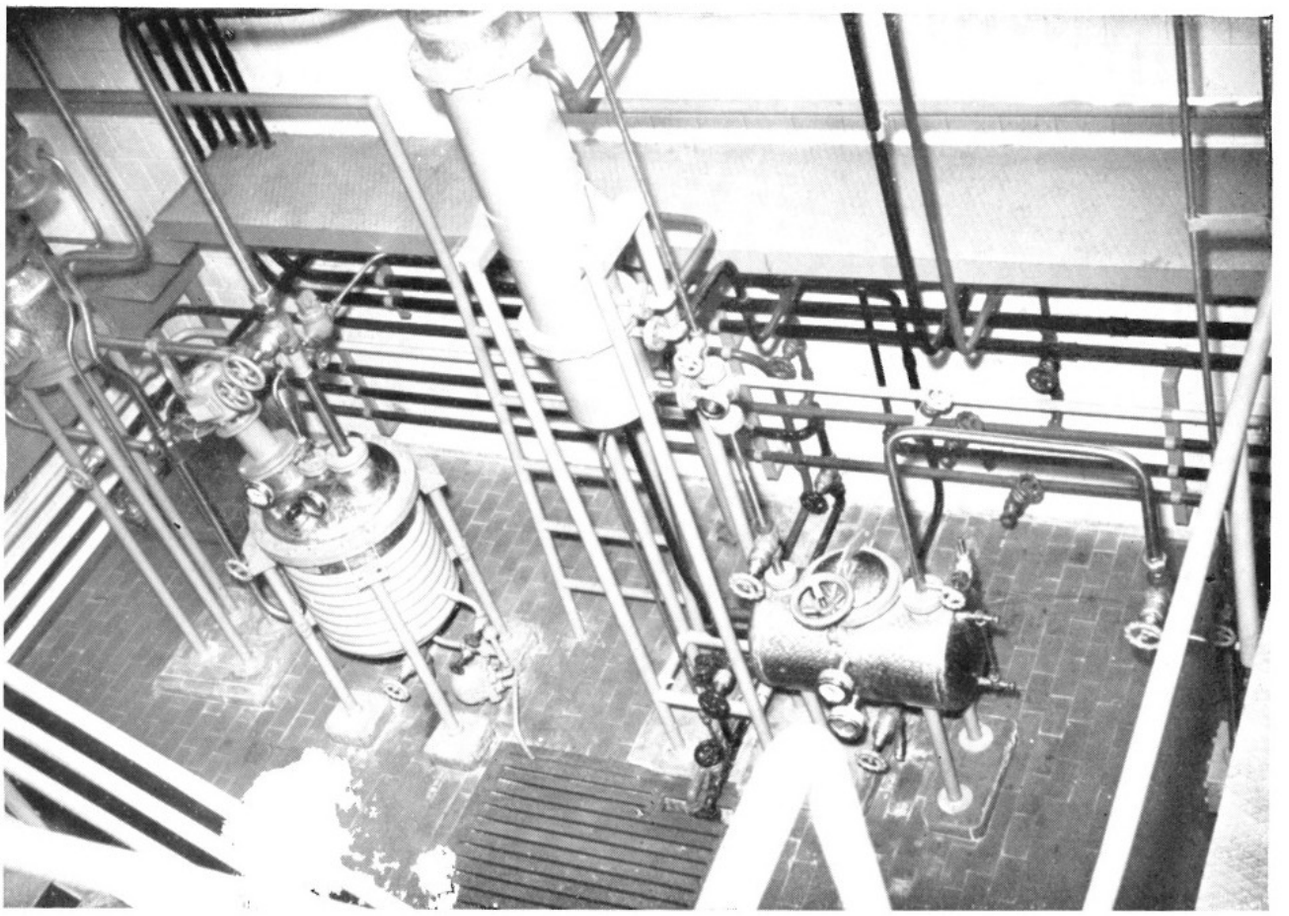

Plate 4. - Solvent recovery plant (G fig. 1).

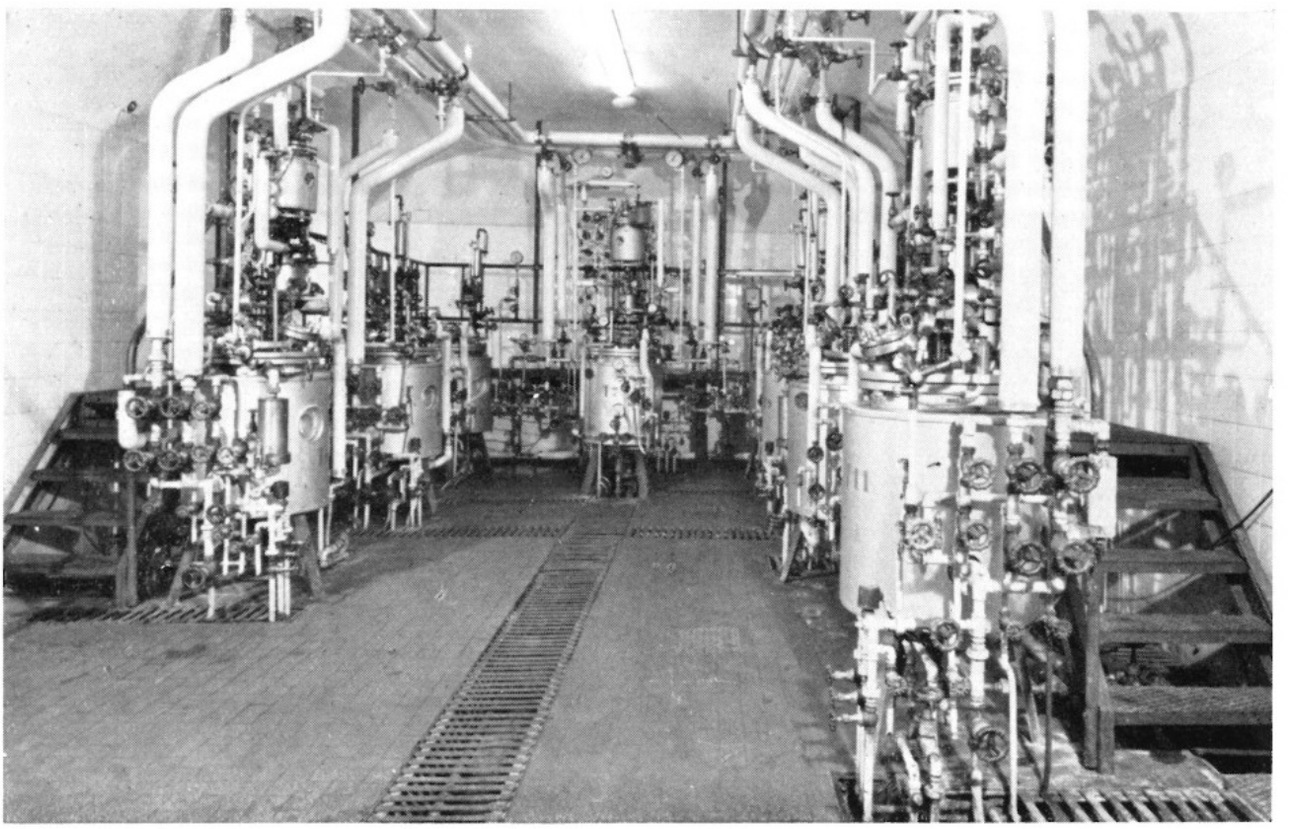

Plate 5. - Group of eight 90 l fermenters.

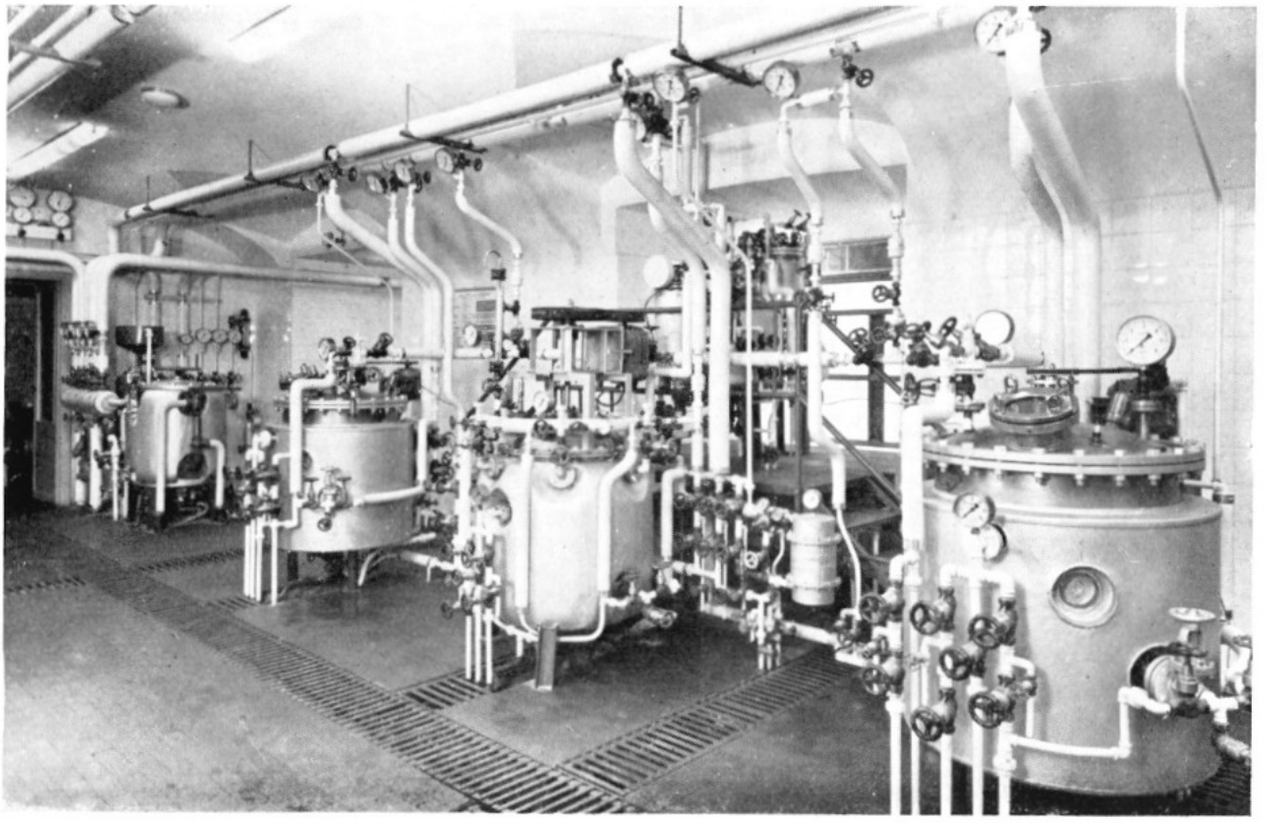

Plate 6. - Group of three 300 l fermenters.

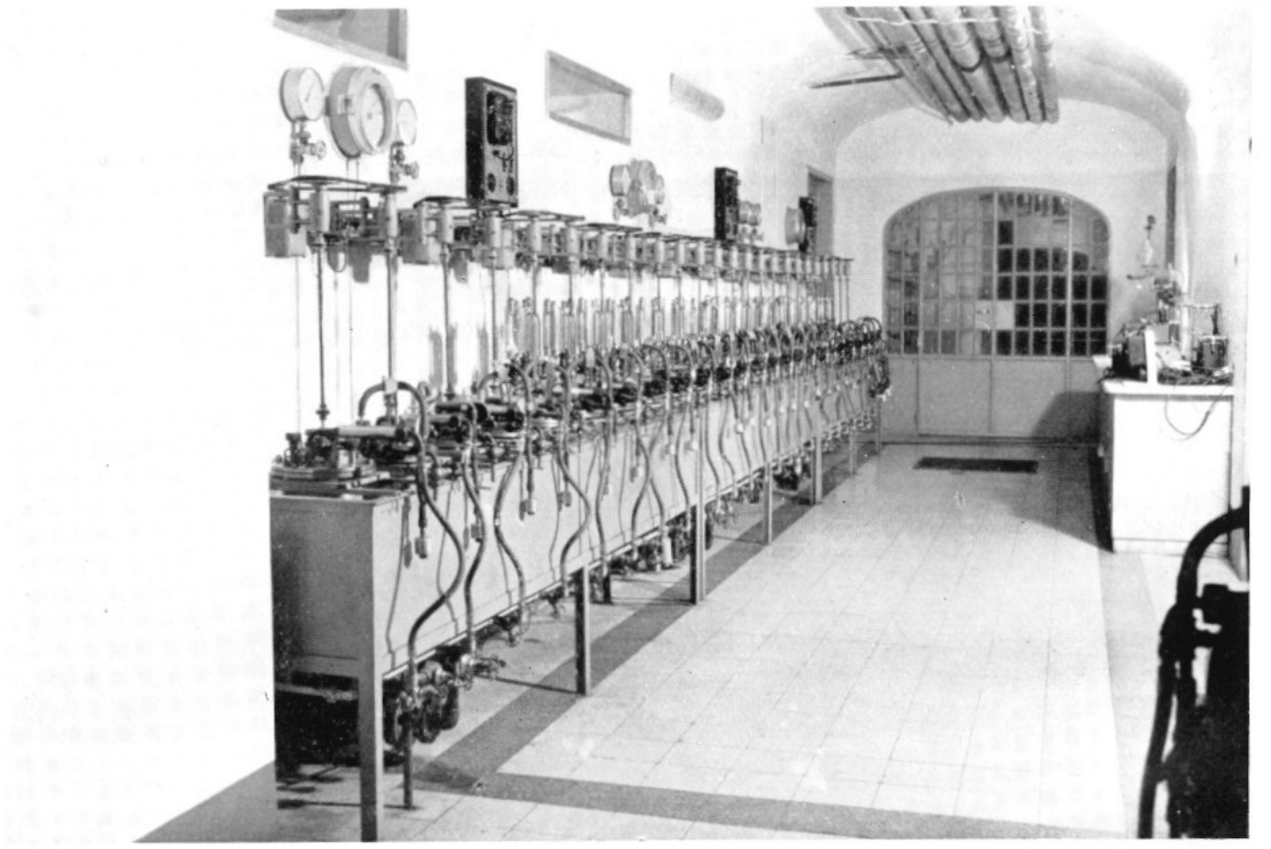

Plate 7. - Three units each comprising eight 10 l fermenters.

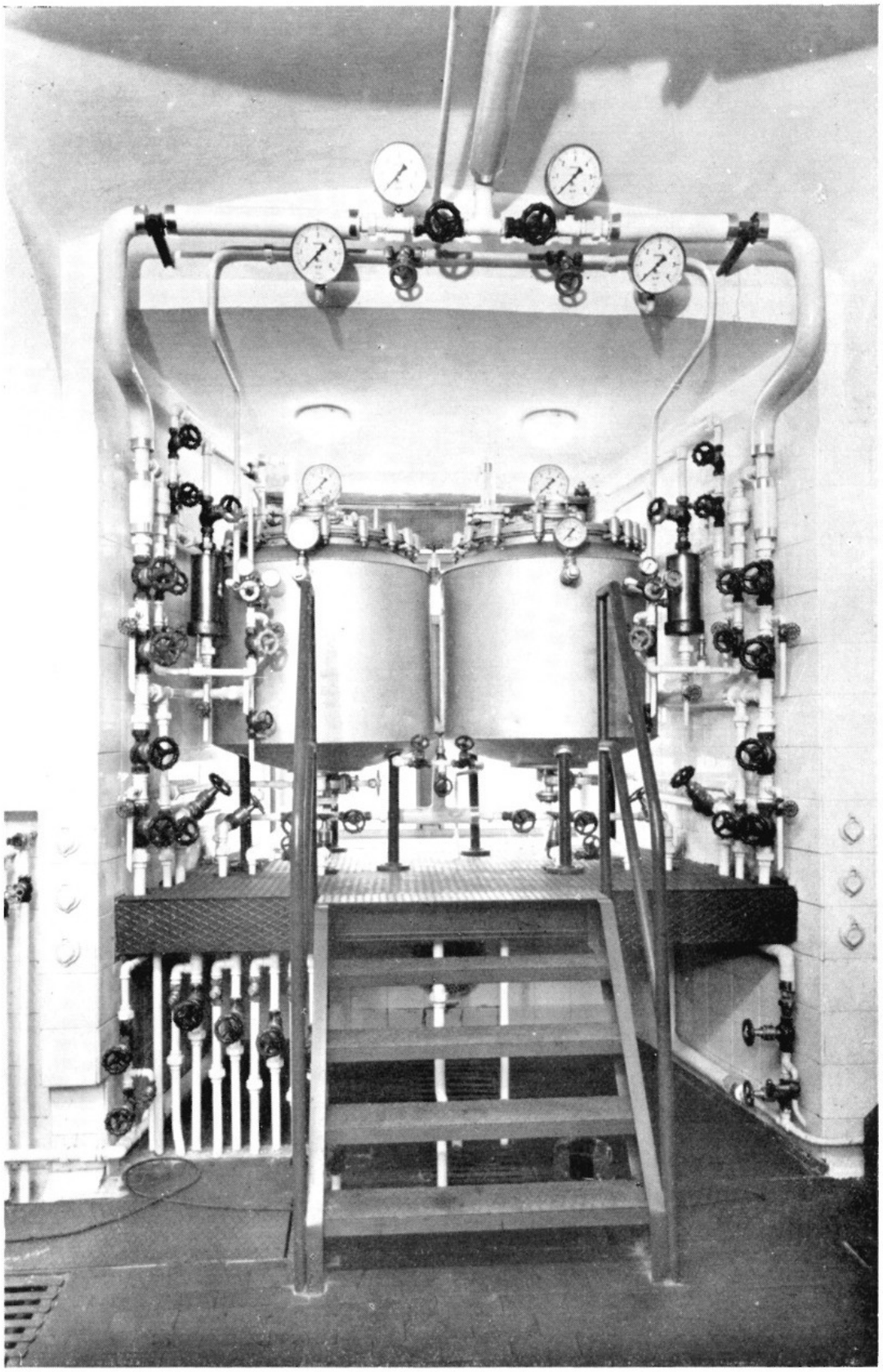

Plate 9. - Group of two sterilisers.

rience showed that they were entirely impraticable owing to excessive formation of rust which was deposited in the fermenters, even during continuous use. In view of this the whole pipe system had to be dismantled and replaced by stainless steel tubes.

Sterile transfer of culture medium from steriliser to the fermenters.

I) Via the measuring vessel. (Example: from ST_1 to T_2, fig. 3-b).

1) In order to transfer quantities of culture liquid up to 10 l from the steriliser to the fermenters an air overpressure of 1.5 atm. is established in the sterilisers by suitably regulating the air pressure through reduction valve R and air outlet valve 40. Of the air and steam group D, valves 30, 32, 33, 34 and 62 are opened, valves 29, 31 and 35 closed.

2) The steam flow through the following points is then interrupted:

a) the lower steam seals of the steriliser and measuring vessel, by closing valves 70 and 71 (steriliser) and 54 and 55 (measuring vessel).

b) all steam seals on the transfer line (inserted for dividing the transfer line into readily sterilisable tracts), marked I, II, III and IV; and valve 80 isolating steriliser ST_1 from steriliser ST_2.

c) the steam inlet to the measuring vessel, by closing valves 51 and 52 and opening security valve 53. Steam present in the transfer line is allowed to escape by further opening of security valve 49.

3) All tracts of the transfer line from the steriliser to the measuring vessel and from the measuring vessel to the fermenter are now prepared for passage of the culture fluid by opening valves 73, 74, 41, 42, 44, 59, the relevant valves of steam seals I and II, and the valves 57 and 37, while cap I on the inoculum port is tightened.

4) When steam has ceased to escape through valve 49 drain valve 72 of the steriliser is gradually opened and culture fluid transfered until the desired volume (measured by a level indicating device described earlier: Paladino et al., 1954) has entered the measuring vessel; valve 72 is then closed.

5) An overpressure of 1.5 atm. is then established in the measuring vessel by opening valve 48 and closing valve 49, the air pressure being regulated by the reduction valve R.

6) The overpressure in the fermenter is then reduced to 0.5 atm., by regulating valves 62 and 40, and the transfer of culture fluid from the measuring vessel effected by opening valve 39. If quantities of culture fluid exceeding the capacity of the measuring vessel have to be transferred to the fermenter, the measuring vessel is filled up with fresh solution and all the transfer operations have to be repeated.

7) After termination of the final transfer all steam seals on fermenters, measuring vessel and transfer line are reestablished, the air pressure in steriliser and measuring vessel reduced, and that in the fermenter increased to the normal working overpressure.

II) Direct. (Example: from ST_1 to T_4 fig. 3-b).

1) An overpressure is established in the steriliser as described above under I 1).

2) The steam flow through the following steam seals is interrupted:

a) the lower steam seal of the steriliser, by closing valves 70 and 71,

b) the lower steam seal of the fermenter; in this particular case by closing valves 41 and 46,

c) all steam seals on the transfer line from ST_1 to T_4; in this particular case by closing valve 77 and the appropriate valves in group VII; at the same time valves 74, 76, 79 and 83 and 84 are closed,

3) Valves 73 and 44 on the transfer line are opened to allow passage of culture fluid,

4) The overpressure in the fermenter is reduced to about 0.5 atm. regulating the air flow through valves 40 and 62, and the transfer of culture fluid effected through the fermenter drain by opening completely valve 45 and regulating the flow of liquid by means of valve 72.

Level marks fixed on the fermenter wall at different heights V_1 to V_4 indicate the volume of culture fluid admitted to the fermenter.

5) After termination of the transfer the drain valves 72 and 45 are closed, the steam seals are reestablished and the valves isolating the steriliser and the fermenter from the rest of the fermentation plant are reopened.

Normal air overpressure is established in the fermenter.

Sterile transfer of the contents of one fermenter to any other fermenter in the pilot plant. (Example: from T_4 to T_2 fig. 3-b).

The pipe system of the pilot plant is arranged in such a way that sterile transfer of the contents of any one fermenter to any of the others is possible. The procedure of the particular case is as follows:

1) An overpressure of about 1.5 atm. it established in fermenter T_4 in the manner described above.

2) The steam flow through the following steam seals is interrupted:

a) the lower steam seals of the fermenters, by closing valves 41 and 46 in T_4 and T_2.

b) the steam seals on the transfer line, i.e. the appropriate valves in groups V, VI, VII and VIII, as well as valves 81 and 83.

3) The air overpressure in fermenter T_4 is reduced to about 0.5 atm. by adjusting valves 62 and 40 and the transfer effected by opening completely drain valve 45 of fermenter T_4 and regulating the flow of liquid by means of drain valve 45 of fermenter T_2. The volume admitted may be estimated from the level indicators (V_1 to V_4) fitted on the fermenter wall.

4) After termination of the transfer, valves 45 of both fermenters are closed and the original conditions established with regard to steam seals and air overpressure.

Services.

The pilot plant is provided with the following services:

1) Steam, 2) Cold water, 3) Hot water, 4) Compressed air, 5) Cold brine, 6) Gas 7) Electricity.

1) *Steam.*

The lay-out of the steam service is illustrated schematically in fig. 4. Saturated steam arrives in the pipe lines in the different rooms of the pilot plant at a pressure of 3 Kg/cm². Two boilers for steam generation are installed. One of these, a naphtha burning boiler, provides a continuous steam supply during the fermentation for the maintenance of the steam

seals on all pilot plant fermenters. The other (Plate 6 extreme left) electrically heated, is used periodically in addition to the naphtha burning boiler for steam supply for the sterilisation of culture fluid in the fermenters. In case of emergency it can replace the naphtha burning boiler. With all fermenters in action the continuous steam consumption amounts to about 130 Kilos at a pressure of 3 atm per hour.

The sterilisation of culture media and fermenters is carried out with 150 Kilo-hours of steam at 3 atm. pressure. This allows the sterilisation in 30 to 40 minutes of 400 l of culture medium distributed in the pilot plant fermenters in any desired quantities.

The usual sterilisation practice is to sterilise at one time a group of two 300 l fermenters each containing 200 l of culture medium, or a group of four 90 l fermenters each containing 50 l of culture medium.

The laboratory 10 l stainless steel fermenters are sterilised in batches of eight in a special autoclave housed in the room for media preparation (M fig. 1). The steam consumption for the sterilisation per batch is 150 kilos of steam at 3 atm. pressure when the autoclave has to be heated from room temperature; when the autoclave is at 80° C about 40 kilos of steam are required.

2) *Cold water.*

Cold water is available at a temperature of 7-18° C depending on the season.

Continuous cold water supply is required for the production of steam, and thermoregulation of the fermenters. The cooling of the air compressors is effected by a special closed circuit of distilled water. Additional amounts of cold water are used periodically for the cooling of the fermenters after sterilisation, the preparation of culture media, the preparation of cold brine, the cooling of the cold brine compressor and cleaning operations (for diagram of water circuit see fig. 5).

The temperature of the fermentation room varies normally between 30° and 35° C.

The maximal cold water consumption at the hottest season for all the above indicated uses is about 3000 l per hour. In the Winter season it is reduced by about 10 per cent.

3) *Hot water*

The waste steam leaving the pilot plant is passed through a heat exchanger which thus provides a supply of fresh hot water at a tem-

perature of 40-70° C. It is utilized for maintaining temperatures above 28° C in the fermenters and for cleansing operations.

4) *Compressed air.*

Two water cooled piston compressors are used alternately. Each has a capacity of 1000 l of air per minute at 3 Kg/cm². The air circuit is illustrated in fig. 6.

5) *Cold brine.*

A cold brine circuit (fig. 7) is provided to cool the fermentation mixture in the fermenters to +2° C. 1000 l of cold brine are available, and the compressors have a cooling capacity of about 10.000 kcal/hour.

6) *Gas.*

Gas is consumed for the sterilisation of the sample tube outlet in the 10 l laboratory fermenters, and the sterilisation of the ends of the different inlet tubes for the addition vessels of the pilot plant fermenters.

The average gas consumption is about 2000 l per day.

7) *Electricity.*

a) Lighting circuit.

For illumination of the rooms a single phase A.C. line of 130 volts 50 cycles is used, while for safety reasons for the illumination of the interior of the fermenters a low voltage supply is used, obtained from the normal lighting circuit through a 24 volts transformer.

b) Power circuit.

The power supply is a three phase A.C. line of 225 volts 50 cycles. A special line, carrying up to 1000 amps. is provided for the compressors, the electric boiler and an electric welding machine which is frequently used for repairs and modifications to the pilot plant. The auxiliary electric boiler consumes 120 Kw.

With all motors of the pilot plant in motion, including those of the air and cold brine compressors, the electricity consumption is about 30 Kw per hour.

To this value the power consumption for the electric boiler has to be added.

Accessory services.

Preparation room.

The preparation room (Plates 3 and 3-a) serves for working up culture fluilds obtained in the fermentation room. It contains the same services as are available in the fermentation room. The service pipe lines are placed around all walls of the room just below ceiling level and are provided at intervals of about 2 m. with outlets to which rapid hose couplings can be fitted. The preparation room contains filters of different types (a filter press, a Sparkler filter and a small rotary Bird-Young vacuum filter) for the removal of mould mycelium and two large Sharples centrifuges for the collection of bacteria. It is also provided with a small Podbielniak counter-current extractor (n. 6000) for extraction of aqueous solutions with organic solvents. Furthermore it contains a number of stainless steel jacketed vessels of different capacities (25 l, 50 l, 200 l and 500 l) in which solutions can be heated to 100° C or cooled to +2° C by connecting the jackets by means of flexible rubber hoses provided with rapid couplings to the outlets of the steam or cold brine services. The fermentation liquids may be pumped from the fermenters in the fermentation room into these containers or directly to the filters. All electrical equipment in the preparation room is spark-proof.

Room for media preparation and sterilisation of laboratory fermenters.

This room (M, fig. 1) contains an horizontal autoclave of 1000 l capacity provided with rails into which a rack containing eight 10 l laboratory fermenters may be rolled from a trolley. (See Chain et al., 1954 Plate 9). It is heated with steam generated in the naphtha burning boiler. The room further contains a dry steriliser of about 500 l capacity, a bench for the preparation of culture media, a stainless steel sink with three divisions for the washing of glass ware, and an electrically heated and ventilated drying cupboard.

Solvent recovery plant.

This plant contains conventional distillation equipment made of stainless steel for the recovery, rectification and vacuum distillation of organic solvents in semiindustrial quantities. The capacity of the

distillation kettle is 100 l, the fractionation column has 28 theoretical plates. The installation of a climbing film concentrator for aqueous solutions is in progress.

Organisation of the personnel.

The proper organization of service personnel is an essential condition for the proper functioning of the pilot plant. The personnel of the pilot plant consists of technicians with different training (electricians, mechanics, plumbers) who work in three 8-hours shifts. Two persons per shift are sufficient for carrying out the necessary routine plant controls with all the fermenters in function. Extra personnel for the preparation of media, recovery of fermentation products washing of glass-ware and general cleaning is provided. The pilot plant and its personnel are supervised by two foremen. A well equipped workshop is available for maintenance, repairs and development; It is staffed with four mechanics and one foreman. In most metabolic studies of microorganisms chemical and biological analyses of different types have to be carried out on the culture fluid at regular intervals. It is therefore essential to have at the disposal of the research workers a well functioning analytical unit for chemical analyses and microbiological assays. The number of personnel in this unit depends, of course, on the number of analyses to be completed. With regard to the simple normal routine chemical analysis of culture media constituents, such as sugars, nitrogen, volatile acids etc., it may be assumed that in general one technician cannot conveniently carry out more than 20 to 30 analyses in duplicate per day of any one variable. That is to say, in a fermentation run where samples have to be taken twice a day from each fermenter one technician would not be able to deal satisfactorily with more than 10-15 fermenters for the determination of one single variable. If three variables in the culture fluid have to be determined by chemical methods a minimum of three technicians would be required for each unit of 10-15 fermenters. This number is, of course, necessary only if the fermentations are to be run continuously, without a break of a few days between one fermentation and the next, or if the analytical samples cannot be accumulated because of the instability of the variables to be measured.

For the biological assay of antibiotics by diffusion methods one technician can deal with up to 48 samples per day.

Roma — Istituto Superiore di Sanità - International Research Centre for Chemical Microbiology.

REFERENCES

(1) Chain E. B., Paladino S., Ugolini F., Callow D. S. and Van der Sluis J. - (1954) Rend. Ist. Sup. Sanità, *17*, English Edition p. 61.

(2) Dion W. M., Carilli A., Sermonti G. and Chain E. B. - (1954) Rend. Ist. Sup. Sanità, *17*, English Edition p. 187.

(3) Fortune W. B., McCormick S. L., Rhodehamel H. W. and Stefaniak J. J. - (1950) Ind. Eng. Chem., *42*, 191.

(4) Jacobs W. L., Wright R. K. and Hildebrandt F. M. - (1948) Ind. Eng. Chem. *40*, 759.

(5) Paladino S., Ugolini F. and Chain E. B. - (1954) Rend. Ist. Sup. Sanità, *17*, English Edition p. 87.

(6) Pfeifer V. F., Vojnovich C., and Heger E. N. - (1952) Ind. Eng. Chem. *44*, 2975.