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DEMOCRATIC PEOPLE'S REPUBLIC OF KOREA

Technical report: Evaluation of Sunchon Pharmaceutical Plant Antibiotics Division and Suggestions for Improvement*

Prepared for the Government of the Democratic People's Republic of Korea
by the United Nations Industrial Development Organization
acting as executing agency for the United Nations Development Programme

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United Nations Industrial Development Organization
Vienna

* This document has not been edited.

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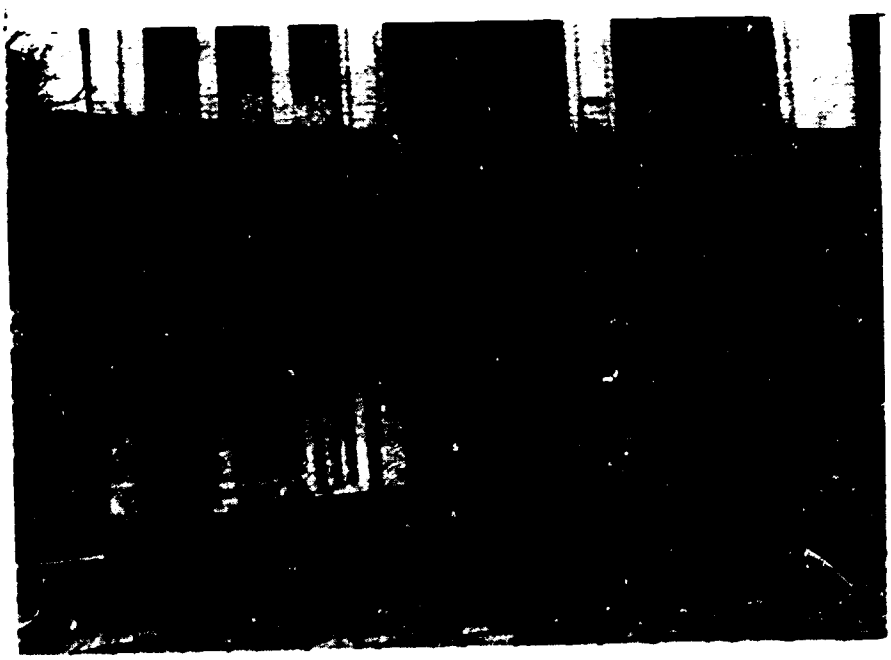
Evaluation of the Sunchon Pharmaceutical Plant Antibiotics Division and suggestions for improvement.

In the course of initiating the project work, discussions were held with the Vice Director of the General Bureau of Pharmaceutical Industry, Mr. Kim Kwang Hum. A detailed plan of work shedule was chalked out and accepted by both of us. The Pharmaceutical complex is 80 Km north of Pyongyang. As the Hotel facilities were not very good in the local area, it was suggested by the officials, that we go to the plant every day in the morning and return in the evrning. We were assisted in the discussions by Mr. Yun Ho, Manager from the Bureau, who acted as the interpreter and guide.It was also agreed upon that all drawings, flow sheets and other essential details willbe made available for the work and discussion. Mr.Kim Kwang Hum was with us during the entire period of the work, during the factory visit.

On reaching the factory we were received by Mr. Sing Yong Uk, Chief Engineer, as the Managing Director Mr. Sin Sok Chan was abroad on official work. Mr.Chang Ryong Hin, Pricipal Engineer for technical development assisted us from the factory during the visits and discussions.

Pharmaceutical complex - near entrance



Entrance - Fermentation block

1. General

The Antibiotic plant is part of the Pharmaceutical complex at Sunchon. The complex works under the Ministry of Public Health. Sunchon is a small town about 80 Km north of Pyongyang. The roads are motorable, though many parts which are under widening need repairs. The aproach road to the factory from one side cutting across railway lines is accessible mainly for trucks. During monspon seasen it will be a slush. From the opposite side the roads are alright. The plant was commissioned in 1958, and reminds us of its ancient past in many ways.

- Total area .. 220,330 M^2
- Building area .. 32, 450 M^2
- Total employees .. 2100
- Total technicians .. 400
- Engineers .. 140
- Fermentation area .. 10885 M^2
- Personnel for Fermentation & Purification .. 266
- Others products manufactured:-

  Asprin

  Liquid injectibles like Glucose, Gentamicin, Magnesium sulphate, Vitamin C etc.

  Bottles and ampules for the above.

2. Penicillin production .

2.1. Culture and laboratory work.

2.1.1. The culture produces an average yield of 18,000 to 20,000 units/ml (10.8 to 12 gms/lt). The international level at present can be put in the range of 60,000- 65,000 u/ml (36 -39 gm/lt) The culture is maintained on Korean millet, the grains of which appears to be quite smaller in size in comparison with millet found in other countries.

2.1.2. The culture is grown in Roux bottle in the following medium.

a) Molasses .. 1.5% w/v
b) Peptone .. 0.6 ,,
c) Glycerine .. 1.5 ,,
d) Calcium chloride.. 0.05 ,,
e) Sodium chloride .. 0.5 ,,
f) Magnesium sulphate .. 0.5 ,,
g) Potassium di hydrogen phosphate .. 0.03 ,,
h) Trace salts
i) Agar .. 2.0 ,,

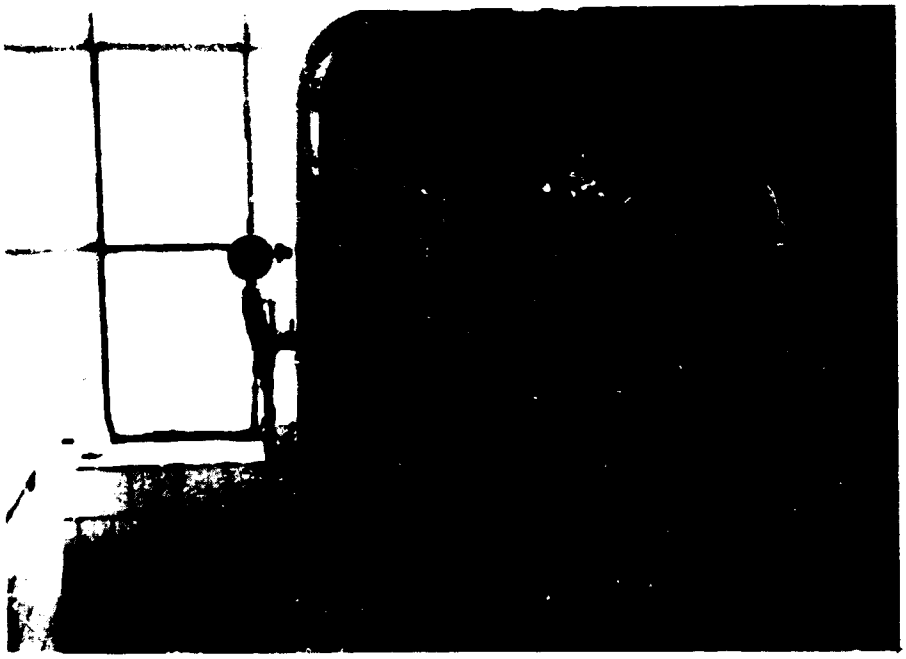
Though in a weeks time the culture spreads out in its typical fungal colony, there appears to be very little amount of spores produced in this period . It is advisable to increase the time of incubation for two weeks depending upon the dryness of agar in the bottle.

2.1.3. In 250 ml flask,Korean millet 10 gms and 8 ml of water are boiled and then sterilized at 120°C for 30 minutes and used for the cultivation of spores.

2.1.4. The fungus grown on the Roux bottle is scraped with 100 ml of sterile water and, 2 ml of this is distributed to 50 flasks of millet as explained in 2.1.3.The flasks with the fungal spores are incubated at 25°C for 7 days and used to inoculate the I stage Inoculator tank of 400 lt working volume. These spores on millet flasks are stored at room temperature for 6 months.

2.1.5. The shaker room is provided with one obsolete shaker, to hold 250 ml and 500 ml flasks. The temperature control is through 'open wire' heaters. This is very primitive and does not confirm to any safety standards. The ambient temperature in the room will

Penciilin - culture



Autoclave

be uneven as there is no air circulation. During summer cooling is done through Methanol in coils. All the equipment are in a dilapitated state. Acurate temperature controllers with fan coil units and better shakers are a must.

2.1.6. Inoculation of flasks is done in an inoculation room. There is one UV light and the bench is cleaned with phenolic swab before microbiological work. Provision of laminar flow hood with sterile air flow and one more UV light are a must. This will improve the methodology and GMP standard.

2.2. Inoculator tank.

2.2.1. There are 3 stainless steel 1000 lt inoculators (I seed tank). The quality of the metal is very poor. Each of these inoculators is provided with 2.8 KW motor for agitation. The agitator shaft is driven by pulley block belt drive assembly. The vessel is crude and rough on the inside with coatings on the inside. There is an iron ladder to go in to the vessel. There are 3 baffle plates, 3 agitator blades with curved vanes. There is an antifoam blade on top. There is 25 mm single hole sparger for aeration. The jacketed vessel is clad in glasswool for protection against ambient heat shock.

2.2.2. The vessel design has the following dimensions.

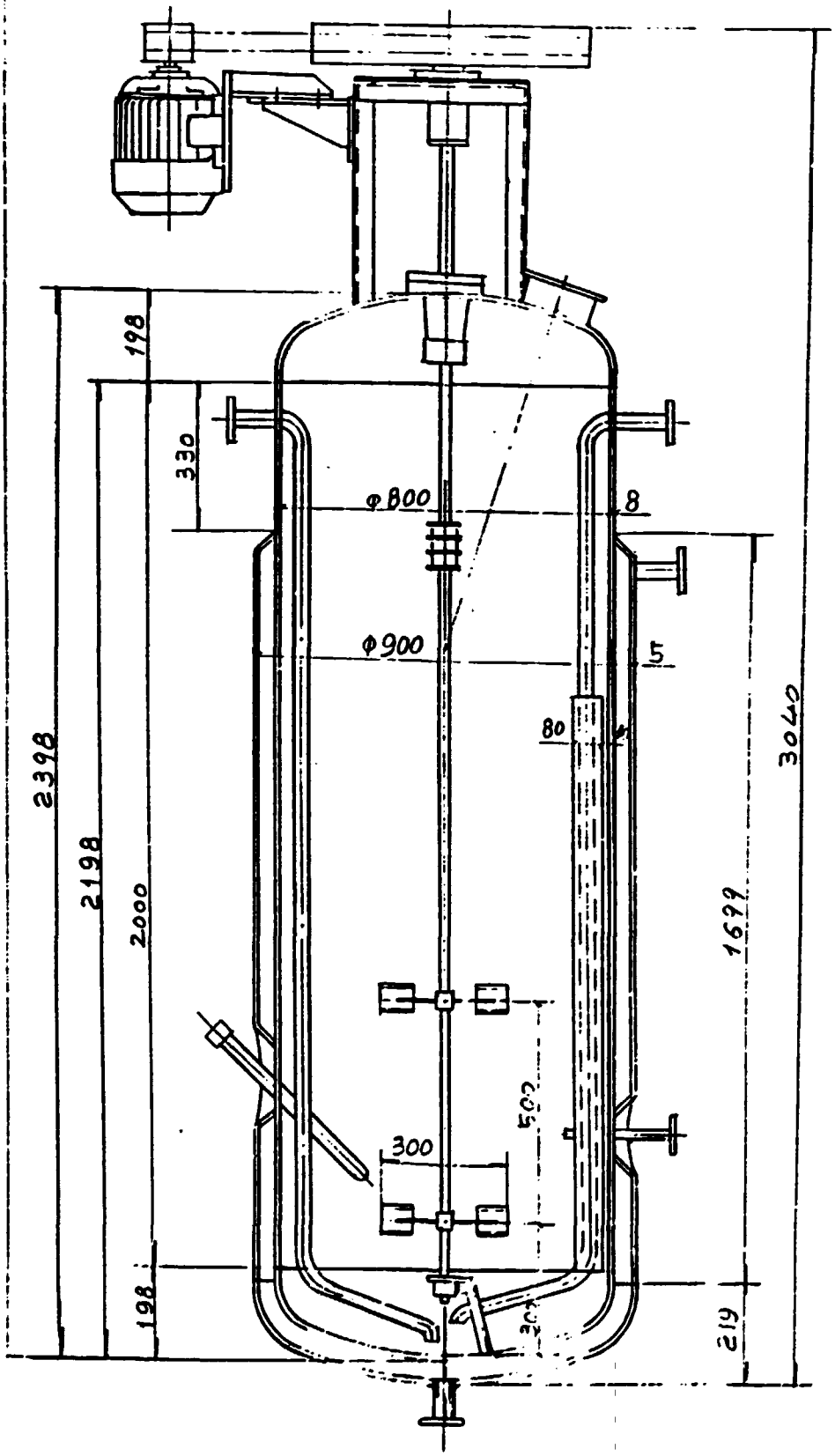
a) Vessel height .. 2200 mm
b) Vessel diameter .. 800 mm
c) Agitator blade diameter .. 300 mm
d) Thickness of Vessel .. 8 mm
e) Jacket thickness .. 50 mm
f) Baffle breadth .. 80 mm
g) Manhole with rubber gasket.
h) Total volume .. 1106 lts.
i) Working volume .. 400 lts.

2.2.3. The air filter is made out of mild steel. It is filled with cotton on the top and bottom and activated carbon in the middle. The filter is sterilized with steam prior to media sterilization. Though there is a jacket there is no steam connection .

2.2.4. Medium composition.

a) Soyabean flour .. 5% w/v
b) Cane sugar .. 3% ,,
c) Calcium carbonate .. 0.3% ,,

Penicillin — Inoculator



1000ℓ 접종물 탱크

d) Potassium dihydrogen phosphate .. 0.2% ,,
e) Sodium thio sulphate .. 0.1% ,,
f) Sodium sulphate .. 0.05% ,,
g) Magnesium sulphate .. 0.025% ,,
h) Ammonium nitrate .. 0.125% ,,
i) Spyabean oil .. 0.1%
pH 6.5

2.2.5. Medium sterilization.

The medium components are brought to the vessel, mixed and the volume is made up to 400lts.Then steam is fed in the jacket of the vessel and with continuous stirring it is heated to 100°C. Then direct steam is fed in to the vessel from air line and the seed transfer line and the temperature is raised to 120°C.The medium is sterilized for 30 minutes. It is then cooled by feeding water in the vessel jacket. The following analysis are made :

a) pH
b) sugar
c) Ammonia nitrogen
d) Phosphorous

2.2.6. Inoculation is done through a metal screw cap on the lid of the manhole. An alcohol flame is put around the cap. The vessel is depressurized and the millet spores are inoculated. The sampling device through a pipe with steam connection is crude and needs modification. Semi sterile zone with steam and cap should be provided.

2.2.7. The parameters.

a) Temperature .. 25°C
b) Agitator rpm .. 220
c) Aeration .. 600 lpm
d) Over pressure .. 0.2 kg/ cm^2
e) pH before seeding .. 6.8
f) pH during the run .. 6.5-6.8
g) Age .. 48 Hrs

2.2.8. The controlling parameters are done manually . Even the temperature control is done manually. There is a hot water tank under pressure and this water is pushed in to jacket to control the temperature. There is only temparature indicator and no other

system. Ordinary water temparature in winter is 4°C and during summer is 14-16°C.

2.3. Seed tank.

2.3.1. There are 3,carbon steel tanks of 3000 lt volume. The vessel is provided with 7 KW motor for the agitation. The shaft is driven by pulley block belt driven assembly. The vessel is provided with an m.s. jacket and a ladder to reach the bottom of the tank. There is an air inlet line with a single hole sparger as in the inoculator tank. There is transfer cum drain line and inoculum inlet line with steam connection. There are two agitator blades with two stirring vanes. The tank inside is very rough and coated with media material. Due to the lack of bottom outlet 2 to 4 lts water always remain in the vessel.

2.3.2. Vessel dimensions.

a) Vessel height .. 2210 mm
b) Vessel diameter .. 1300 mm
c) Jacket thickness .. 50 mm
d) Agitator blade diameter .. 500 mm
e) Baffle breadth .. 130 mm
f) Total volume .. 2935 lts
g) Working volume ..1500 lts
h) Liquid height .. 1136 mm

The sparger should converted to ring sparger and agitator blades are to be designed to rushton flatblade of 580 mm diameter. The individual churning blade should be of length 145 mm and breadth 116 mm. The inside surface is bad and appear to be corroded in some places. At least 2 mm thick stainless steel sheet clading is essential to avoid metallic contamination and to avoid pockets on the surface which may lead to non sterility.

2.3.3. The air filter desigh is the same primitive type in this case also. The jacketed air filter is packed with cotton on both top and bottom and with activated carbon in the middle.

It is imperative that in order to avoid heavy pressure drop and wet cotton filters, they should be changed to glass wool filters or absolute cartridge filters.

2.3.4. Media composition.

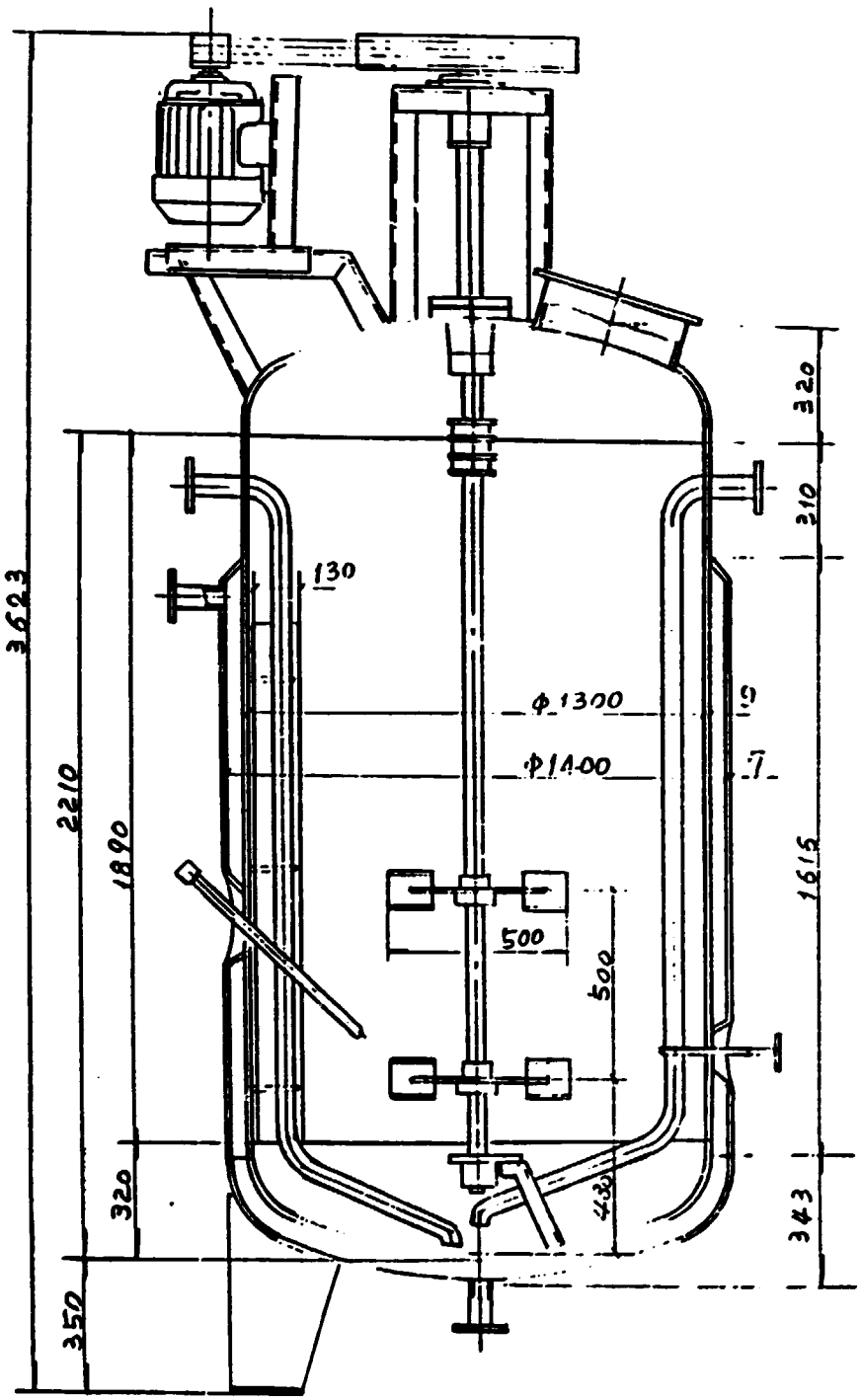
a) Soya bean flour .. 3% w/v
b) Cane sugar .. 1 ,, ,,

Incubator

Seed tank

D2

Seed Tank



3000ℓ 종기탱크

c) Calcium carbonate .. 0.6 % w/v
d) Potassium di hydrogen phosphate .. 0.2 ,, ,,
e) Sodium thio sulphate .. 0.1 ,, ,,
f) Sodium sulphate .. 0.05 ,, ,,
g) Magnesium sulphate .. 0.025 ,, ,,
h) Ammonium nitrate .. 0.125 ,, ,,
i) Soya bean oil .. 0.1 ,, ,,
j) Phenyl acetic acid .. 0.1 ,, ,,
pH 6.5

2.3.5. Medium sterilization.

There is one 3 M^3 steel mixing tank for medium mixing for both seed tanks and fermenters. All the ingredients of the medium are mixed together. The ingredients calculated to 9 M^3 or 12 M^3 are mixed here to make 3 M^3, which is very low. There are good chances that the soya bean flour form grits. It is appropriate to sieve the soya bean flour through a 500 μ sieve. An on line sieve in the pipeline to the fermenter will also remove big grits. Otherwise a wet grinding ingester has to be introduced. Medium is pushed under pressure from the mixing tank. There is a transfer line up to the fermenter bay. The end of the line is connected with a rubber hose pipe which is to be taken to individual fermenters or seed tanks. The medium 1.25 M^3 is heated through the jacket with continuous agitation up to 100°C . Then direct steam is ingested in to the medium through the transfer lines and air line. The temperature is then raised to 121°C and maintained for 30 minutes. The medium is then cooled by passing water in the jacket to the required temperature. One 6 point recorder is equiped to check and record the sterilization temperature of inoculators and seed tanks.

2.3.6. Seeding is done through the seed transfer line. 250 lts are transfered to 1.25 M^3, to make 1.5 M^3 seeded volume. (20%). Sampling is done through same transfer line with steam connection. This looks very out dated. A separate sample line with steam connection is to be provided or the existing line is to be modified as under. The line is to be cut off on the top portion and a reducer with 8 mm ss pipe can be welded to this pipe. An alcoholic swab flame should be provided during the sample collection in order to ensure sterile sample for proper analysis.

The following analysis are done for the broth sample .

a) ...

b) sugar

c) ammonia nitrogen

d) phosphorous

2.3.7. The parameters.

a) Temparature .. 25°C

b) Aeration .. 1050 lpm.

c) Agitation .. 220 rpm.

d) Over pressure .. 0.2- 0.4 Kg/ Cm^2.

e) pH during the cycle .. 6.5-6.8.

f) Running time of seed .. 18 Hrs.

Sterility check is done both in solid agar medium and in broth medium. As it is impossible to have a clear picture before overnight incubation, the seed is transfered on visual observation to the fermenter.

2.3.8. The controlling parameters are minimal as in the inoculum tank. Even temparature control is done manually. Air flow recorder units were found disconnected in some vessels. It is not clear, due to lack of equipment whether any accurate air flow is maintained at all.

a) Temparature recorder controller with an accuracy of $\pm$ 0.2°C is very essential.

b) pH indicator with probe mounted on the vessel is essential.

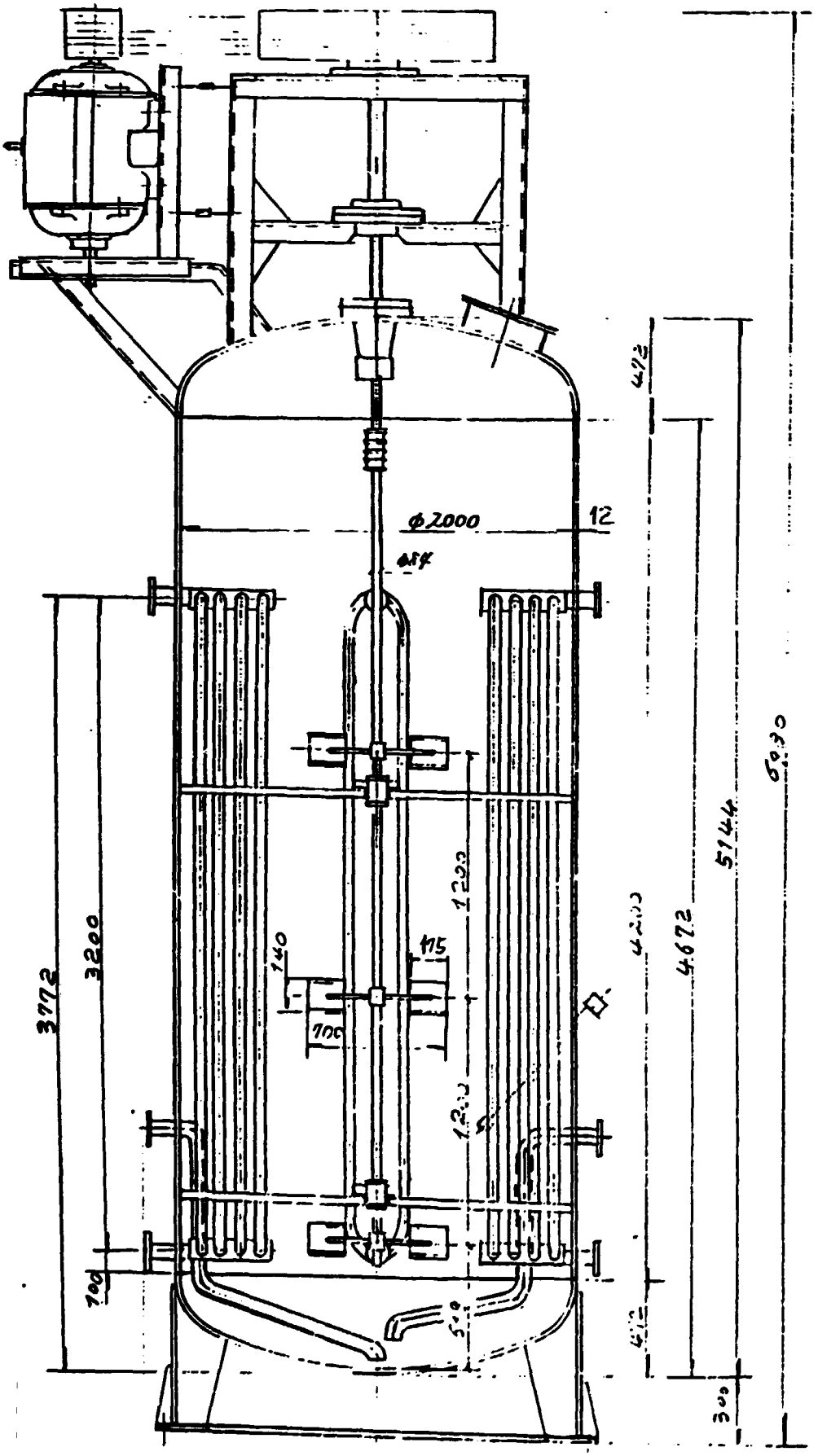
c) Dissolved O_2 monitoring system with probe on vessel is essential.

2.4. Fermenter.

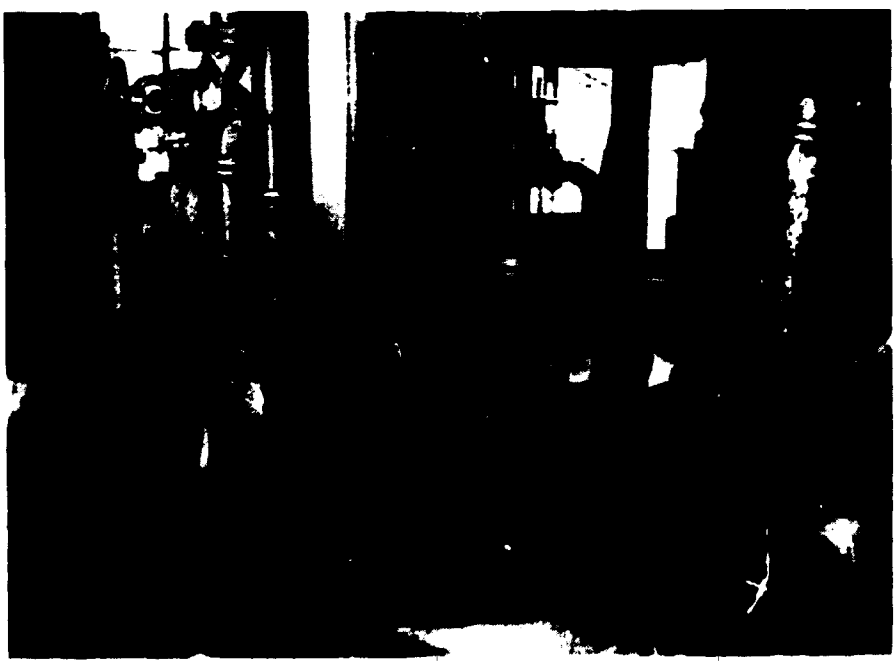
There are 6, carbon steel fermenters of 15 M^3 each and 2, of 20 M^3. The agitator motor is AC, of 40 KW capacity, with pulley block belt drive assembly. There are 4 sets of cooling coils 6 m^2 each inside the vessel. There is a single hole sparger of 10 mm tube at the bottom of the stirrer. There is one seed line, one harvest line. Though there is a sight glass assembly, this has been removed and a blind has been provided. There are crude welding joints on all over the top of the fermenter. The manhole is provided with an ordinary rubber gasket, and is replaced every two months after 10 cycles. The surface inside the vessel is very rough. The shaft seal is made of graphited azbezton and in some cases they do leak. Under this is the bearing assembly. Inside the fermenter the shaft is enclosed in wooden sleeve with casings connected to tierod ends at the sides of the fermenter. At he base there is a matallic sleeve of copper aluminium alloy which is supported

D3

Fermenter

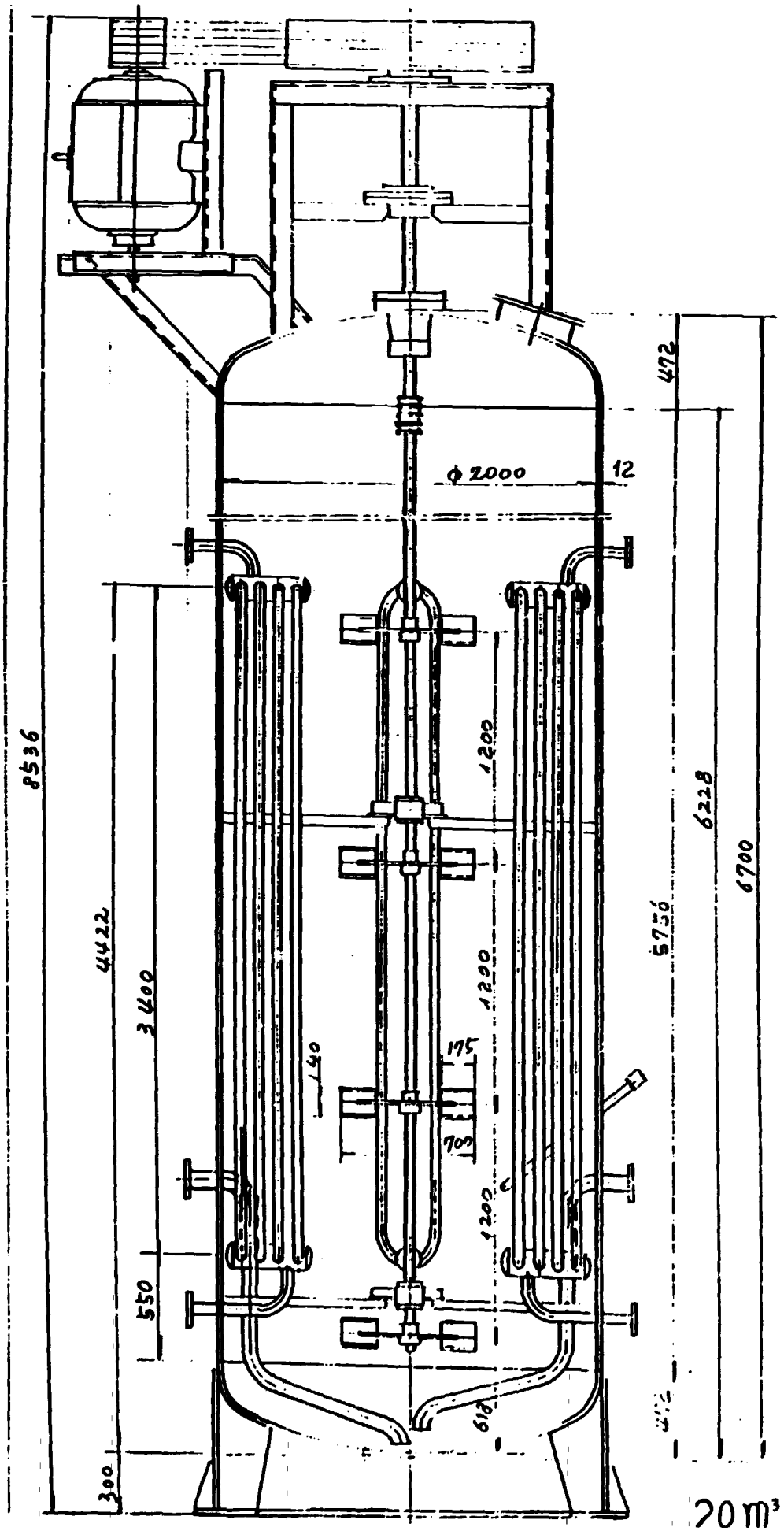


Pencillin Fermenter

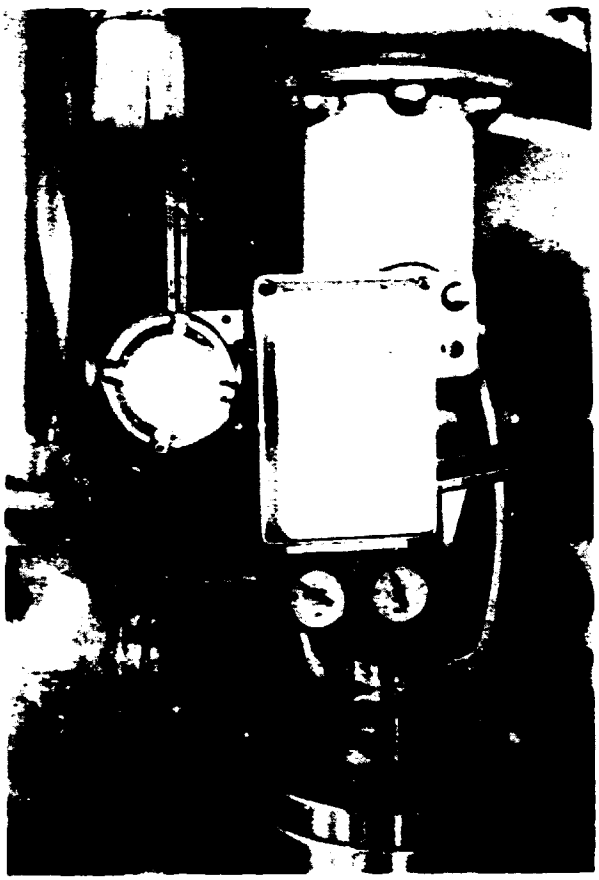


Fermenter

D 4



20 m³ 발효탱크



Pneumatic control

Top cover

Inside view

from the bottom of the fermenter.

2.5. Design of vessels.

2.5.1. Height .. 5144 mm - 15 M^3

2.5.2. ,, .. 6228 mm - 20 M^3

2.5.3. Vessel diameter .. 2000 mm - for both

2.5.4. Agitator blade diameter .. 700 mm

2.5.5. Agitator shaft diameter .. 84 mm

2.5.6. Cooling coils from the bottom of the vessel .. 840 mm

2.5.7. Height of the cooling coils .. 3200 mm

2.5.8. Total volume 15,714 (15 M^3))

2.5.9. ,, ,, 19,573 (20 M^3)

2.5.10. Thickness of vessel plate .. 12 mm

2.5.11. Agitator blades 3. size 175 mm length, 140 mm breadth

2.5.12. 1st blade from the bottom of the vessel .. 618 mm

2.5.13. Liquid height at 10.5 M^3 .. 3344 mm

2.5.14. ,, ,, at 14 M^3 .. 4600 mm

2.5.15. Air is filtered in the same way as in the case of inoculator and seed tanks.

2.6. Medium for the process and parameters.

2 6.1. Composition.

a) Soya bean flour .. 3% w/v
b) calcium carbonate .. 0.4 ,, ,,
c) Potassium di hydrogen phosphate .. 0.2 ,, ,,
d) Sodium thio sulphate .. 0.5 ,, ,,
e) Sodium sulphate .. 0.05 ,, ,,
f) Magnesium sulphate .. 0.025 ,, ,,
g) Ammonium nitrate .. 0.2 ,, ,,
h) Ammonium sulphate .. 0.2 ,, ,,
i) Zinc sulphate .. 0.002 ,, ,,
j) Urea .. 0.05 ,, ,,
k) Oil .. 10 lts.

pH 6.5

2.6.2. The entire components without any carbohydrate source is dumped in the mixing tank. After mixing with ordinary water it is pushed to the fermenter under pressure. Water is added in the fermenter to makeup the volume to 7 M^3. The medium is heated through the coil with steam. When the temparature comes to 100°C, then direct steam is fed in to the fermenter through the air line, seed transfer line

and harvest line. The temparature is raised to 120°C and maintained for 30 minutes. Then the medium is cooled by water supply to the cooling coils.

2.6.3. The fermenter is seeded with 1.5 M^3 seed, seeded volume being 9 M^3. The components initially are also calculated to 9 M^3, which is very low. Analysis of pH, sugar, ammonia nitrogen, phosphoros are done.

2.6.4. Parameters.

a) Temparature is maintained at 25°C. The control is with a pneumatic valve and the water supply is through cooling coils. As the ambient temperature is low, stirring and the reaction in the vessel does not warrant too much of the coolant. There is no heating provision. System is obsolete and require electronic control system.

b) Agitation is kept at 120 rpm.

c) pH is only monitored without any control system.

d) The air supply is at the rate of 1:0.7, which seems very low. There are no control for measuring dissolved O_2 by D.O_2 control system.

e) All assays are conducted only once in 8 Hrs in shifts.

2.6.5. Additions to the fermentation.

There is no initial carbohydrate source in the medium, except for the meagre source given by the defatted soya bean flour

a) There are 2 additional tanks . One of 1.8 M^3 and another of 0.8 M for sugar and phenyl acetic acid(PAA) shots.

b) There are two oil tanks of 1.5 M^3 each. Soya bean oil, whale oil, or sunflower oil what ever is available is utilized.

Sixty % sugar solution is sterilized and kept along with 6.25% PAA. Shots are given hourly, at the rate of 8-10 lts till 108 Hrs and 4-6 lts upto the age of 168 Hrs. Sugar added works uptp 0.05%. Shots are not added on the basis of any analysis. No graphs are made for any running batches.

2.7. Harvest.

The harvest line is steamed along with the steel receiving tank. This is of 20 M^3 capacity. The broth is sent to the tank under pressure. Air is sparged and a pump is operated to circulate the broth in the tank. Fifteen lts of formalin are added to the broth before it is filtered through filter press.

Harvest tank

Overflow receiving tank

Overflow receiving tank

2.8. Suggestions.

2.8.1. Air system.

Air sterilization, as already indicated need drastic modification. Sterilizable cartridge filters (Pall or Ultipore) are the best and are available all over the world. In case if it is not possible for the Company to purchase them, the best other alternative is to get glasswool(emulsified staple fibre). The filters are to be packed with this glasswool. The perseht method of packing with cotton on top and bottom with activated carbon in between is out dated. This packing will lead to enormous amount of pressure drop across the line. After sterilization as there is no steam supply to the jacket of the air filter and the cotton and activated carbon cannot be dried for a long time. There are chances of channelization to occur leading to bacterial contamination.

2.8.2. Air sparger in all the cases is to be modified to ring sparger. A design for the same has been given to the factory personnel.

2.8.3. Agitator blades are to be modified to rushton flat blade turbine, or other model. A basic design has been given. The distance between the blades should be adjusted taking the volume in to consideration.

2.8.4. The support at the bottom of the fermenter for the shaft sleeve, needs modification as it can generate a lot of heat, due to friction. Teflon sleeve with outer matallic covering is very suitable for this operation.

2.8.5. As the harvest line inside the vessel cannot go right upto the bottom, there is always a chance for some water to remain after washing the fermenter. In case the fermenter is not utilized immediately this may harbour enormous amount of bacteria. Each and every time when the vvessel has to be drained, it is essential to close and open the manhole. This is very time consuming and also cumbersome operation. A bottom valve and bottom drain valve with steam and condensate line connection should be provided.

2.8.6. The vent line is open without any scrubbing or steam connection. They allopen in to a tank. The overflow of the broth mycelium is collected and sent as cattle feed to farmers. Water scrubbing is necessary to clean up the vent line. A steam connection is also to be provided. This will kep the line under steam during the time when the vessel has to be kept under pressure.

2.8.7. Media mixing is improper. The soya bean flour in the volume it is mixed is likely to form grits and will be difficult to sterilise. It is advisable to use 500 μ sieve to get rid of the grits. Or wet grinding through an ingest pump is to be carried out.

2.8.8. There is no temperature in dicator on the vessel. Only 6 point recorders are supposed to in commission. Many of them look faulty. The pressure gauge on the vessel may not be the correct indicator for the steam pressure in the vessel. Accurate temperature indicators are very essential.

2.8.9. Parameters.

a) Temperature control should be accurate to ± 0.5°C. This should be carried out with electronic control system.

b) Aeration rate as given to Penicillin fermentation is very low. In the initial stages upto 20 hours when the organism is in the growth phase this may work out. But later on this should be increased to 1:1.2 ratio volume / volume. As there are no measurement of DO_2 or Oxygen uptake rate, the fermentation here is carried out blindly. Vessel back pressure are kept low 0.2 Kg/cm^2, to increase air rate. This should be increased to 0.5 Kg/cm^2.

c) pH indicators for the seed tanks and indicator controllers for the fermenters are essential for the course of fermentation. Any unchecked deviation will lead to disastrous results.

d) Analysis of the broth should be done atleast every 2 hours for Sugar, Ammonia nitrogen, and Activity.and FAA. Depending upon this action should be taken for the additional shots.

e) It is wothwhile to add atleast 1% Lactose and Hydrol in the initial medium, in order not to starve the organism. And checking after 8-12 hours, the shots can be programmed.

2.8.10. As there is a chance of contamination immediately or during the transfer of broth to the holding tank, formalin should be added in the fermenter itself and then transfered to the holding tank. Formalin quantity may be increased to 30 litres.

2.8.11. Production in plant.

a)-Average production in fermenter .. 17,000 u/ml

- Volume at harvest in 15 M^3 fermenter ... 10.5 M^3
- No of batches taken in a year per fermenter ... 36.5
- No of batches run for 6 fermenters ... 219

- Yield perfermenter ... 178.5 mlds
- Total yield for 15 M^3 fermenters... .. 39091.5 mlds

b)- Volume at harvest in 20M^3 fermenters .. 14.5 M^3
- Yield per fermenter .. 246.5 mlds
- No of batches taken in a year per fermenter .. 36.5
- No of batches run for 2 fermenters.. 73
- Total yield for 20M^3 fermenters.. .. 17994.5 mlds
- Total yield for 15M^3 and 20M^3 fermenters .. 57086 mlds
- Yield after purification at 61.5% .. 35107 mlds

c) Suggestions.

i) By improving the harvest volume to 12M^3 and 16M^3 in the fermenters the productivity can be enhanced.

ii) By harvesting 2/2.5M^3 just 18-20 hours before harvest and adding water, feed back inhibition can be reduced and over all productivity will increase.

iii) Procuring high productivity strains will double the output according to international standards.

iv) Use of lactose, hydrol, csl in medium should improve productivity.

v) Sparger, agitator blade modification should enhance productivity

3. Kanamycin.

3.1. Culture and laboratory work.

3.1.1. The culture Streptomyces Kanamyceticus 73 probably from Japanese source, produces on an average 4gm/lt. There is a separate laboratory headed by Dr. Jong u Kyo for the slant preparation, maintenance and seed preparation. Surface growth of slants appears light grey with spores, with no apparent diffusible pigmentation. The culture is not very hard to remove from the slant surface.

3.1.2. The culture slants are grown on the following agar medium.

a) Glucose .. 1% w/v
b) Sodium chloride .. 0.5 ,, ,,
c) Beef extract .. 0.5 ,, ,,
d) Peptone .. 0.5 ,, ,,
e) Agar .. 2.4 ,, ,,
f. pH 6.8–7.0

3.1.3. The slants after they are incubated at 27–28°C for 5–7 days, are kept in the refrigerator at 2–4°C for 2 months. Continuous strain selection are carried out on agar plates. Raw materials are also tested continuously.

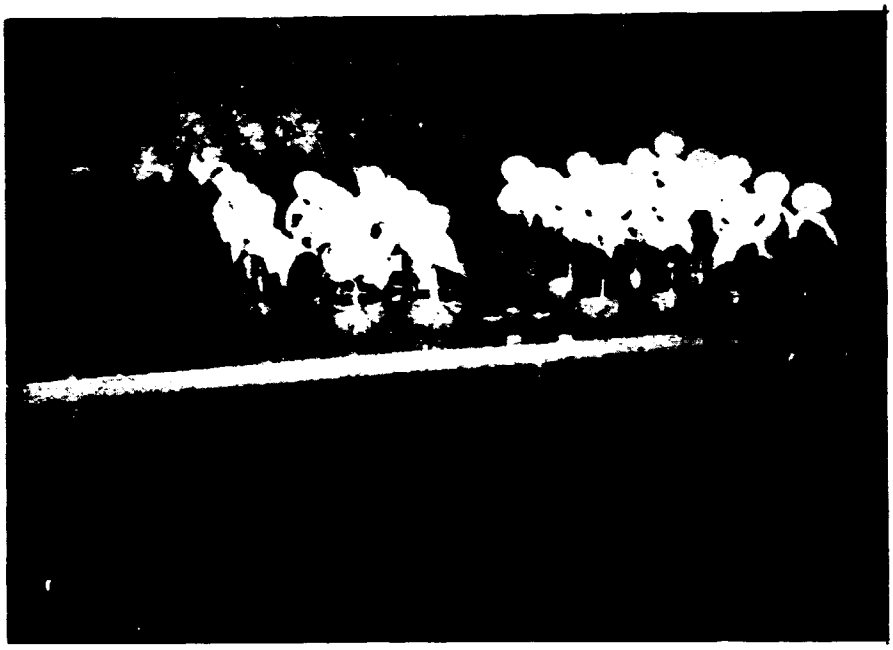
3.1.4. culture preservation.

Sterilized skim milk 1 ml is added on the slant. The spores are scraped with the help of a loop. 0.2 ml of this suspension is taken in sterile ampule and after freezing ,this is hooked on to the lyophilizer. 5 ampules/ slant are obtained this way. The lyphilization is carried out at -70to -80°C, and the ampules are sealed. Sealed ampules are kept at 2-4°C and used to prepare fresh slants as and when necessary. From one lyophil 5 to 6 slants are prepared.

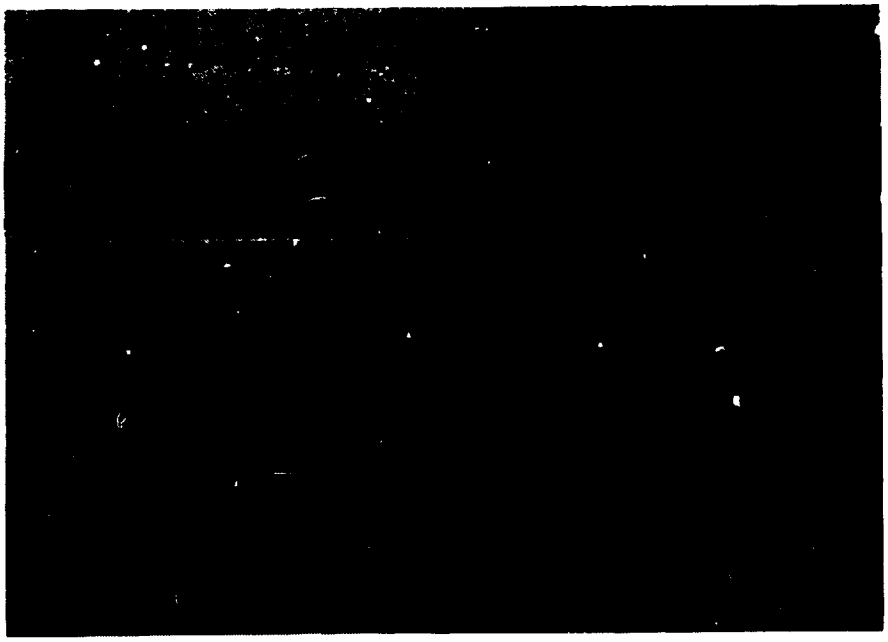
3.1.5. Culture testing in flasks.

Single stage fermentation in shaken flasks is carried out for checking the slants. The top and bottom portion of the slants are not utilized and only the middle portion is utilized. From one slant 15–18 loops can be obtained. The following medium is used in shaken flasks to test the strain.

a) Soya bean flour .. 2.5%w/v
b) Glucose .. 0.5 ,, ,,
C) Corn starch .. 4.5 ,, ,,
d) Sodium nitrate .. 0.8 ,, ,,
e) Potassium di hydrogen phosphate .. 0.05 ,, ,,



Shaker



Centrifuge

f) Zinc sulphate .. 0.01 % w/v

pH 6.3-6.5

The shaker room and inoculation room are exactly as described for Penicillin (2.1.5 and 2.1.6.)

3.1.6. Parameters for shaken flasks fermentation.

a) Temparature .. 27-28°C

b) RPM 240-260

c) Period of fermentation 6 days.

d) Amount taken in flasks 50ml in 750 ml flasks.

e) Medium analysis for pH, sugar, ammonium nitrogen,

Final activity check is done in the central testing laboratory.

3.2. Inoculator tank.

3.2.1. There are three stainless steel 500 lt inoculators.(I seed tank) The vessel design, motor, agitator blade are all like the other tanks.

3.2.2. Vessel dimensions.

a) Vessel height .. 1732 mm

b) Vessel diameter .. 700 mm

c) Agitator blade diameter .. 250 mm

d) Baffle breadth .. 70 mm

e) Total volume approx. .. 630 lts

f) Working volume .. 300 lts.

3.2.3. The air filter is the same as in Penicillin, packed with cotton on top and bottom and with activated carbon in between.

3.2.4. Medium composition.

a) Soya bean flour .. 2% w/v

b) Corn starch .. 2 ,, ,,

c) Glucose .. 0.5 ,, ,,

d) Yeast powder .. 0.125 ,, ,,

e) Calcium carbonate .. 0.2 ,, ,,

f) Sodium nitrate .. 0.1 ,, ,,

g) Sodium chloride .. 0.4 ,, ,,

h) Soya bean oil .. 0.17 ,, ,,

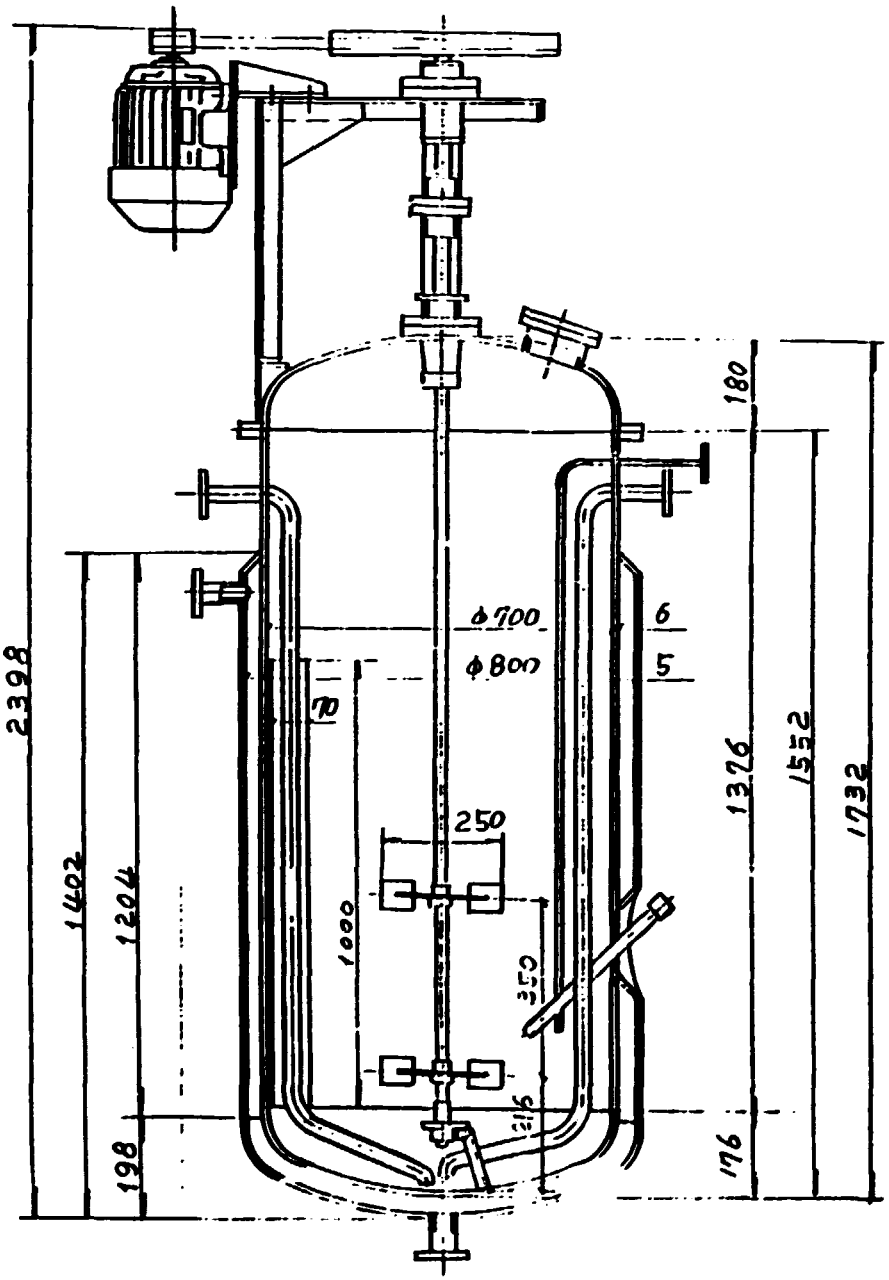
pH 6.5

3.2.5. Medium sterilization is done in the same way as in Penicillin. (2.2.5.) The 6 point recorder is supposed to indicate the sterilization temparature of inoculators and seed vessels.

3.2.6. The inoculation is carried out in either of the following ways.

D5

Inoculator



500ℓ 식종물 탕크

Inoculator

Seed tank

a) The surface growth of one of the slant is scraped and put direct in to the inoculator.

b) In the second method, 20 ml of sterile water is added to the slant, the surface is scraped and then inoculated in to the vessel. The screw cap on top of the vessel manhole cover is kept surrounded by an alcohol swab flame. The vessel is depressurized and the contents of the slants are poured in to the vessel.

3.2.7. The parameters.

a) Temparature... 28°C

b) Air .. 1:0.6-0.7 v/v

c) Agitation .. 220 rpm

d) Over pressure .. 0.2 Kg/cm^2

e) Age .. 50-60 hours

pH 6.5 final 7.8

3.2.8. Only temparature control is carried out, that too manually as is done in Penicillin. There is a hot water tank under pressure to supply water to the jacket of the vessel.

3.3. Seed tank.

3.3.1. There are 3 carbon steel tanks of $3M^3$ and one of $6M^3$ capacity. The general description of the vessel is the same as in Penicillin.(2.3.

3.3.2. Dimensions of $3M^3$ vessel is the same as given earlier(2.3.2.). The $6M^3$ vessel has the following dimensions.

a) Vessel height .. 3200 mm

b) Vessel diameter .. 1600 mm

c) Agitator blade diameter .. 600 mm

d) Jacket thickness .. 50 mm

e) Total volume .. 6400lts

f) working volume .. 4000 lts.

g) Baffle breadth .. 160 mm

3.3.3. Air filters are the same as in Penicillin with the same packing material(2.3.3.)

3.3.4. Medium composition.

a) Soya bean flour. 3% w/v

b) Corn starch .. 4 ,, ,,

c) Glucose .. 0.5 ,, ,,

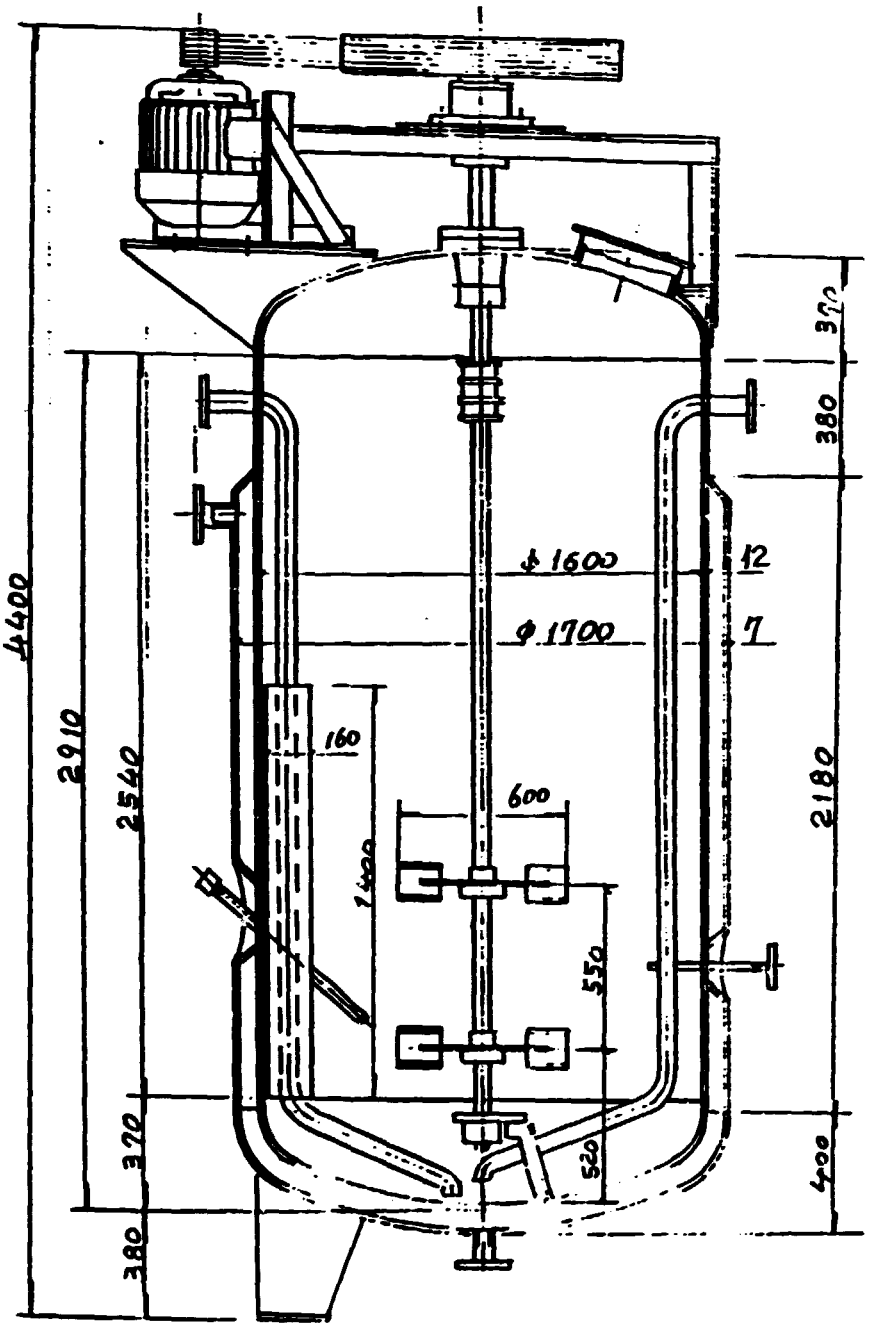
d) Yeast powder .. 0.175 ,, ,,

e) Calcium carbonate .. 0.1 ,, ,,

f) Sodium nitrate .. 0.4 ,, ,,

D6

Seed tank



6000ℓ 종자탱크

g) Zinc sulphate .. 0.01 ,, ,,
h) Soya bean oil .. 0.2 ,, ,,
pH 6.5

3.3.5. Medium sterilization is done in the same way as in other seed vessels.(2.3.5.) It is sterilized for 30 minutes at 120°C.

3.3.6. Seeding is done through inoculum transfer line. 300 lts are transfered to make up to 1.5M^3 seeded volume. Sample analysis are done for:

a) pH
b) Sugar
c) Ammonia nitrogen

3.3.7. The parameters.

a) Temparature .. 28°C
b) Air .. 1000 lpm
c) Agitation rpm 220
d) Over pressure .. 0.2-o.4 Kg/cm^2
e) pH initial 6.5, final 8.0
f) Running time of seed .. 32-36 hours

3.3.8. Acurate temparature control, air flow control, pH indication are essential. DO_2 measurement and control are essential as there is no indication of the O_2 requirement at any time.

3.4. Fermenter.

There are 2 of 15M^3, 2 of 20M^3, and 1 of 35M^3; carbon steel fermenters. The description of the fermenter is the same as in Penicillin.(2.4.) The fermenter appears a little more clean and neat in workmanship.

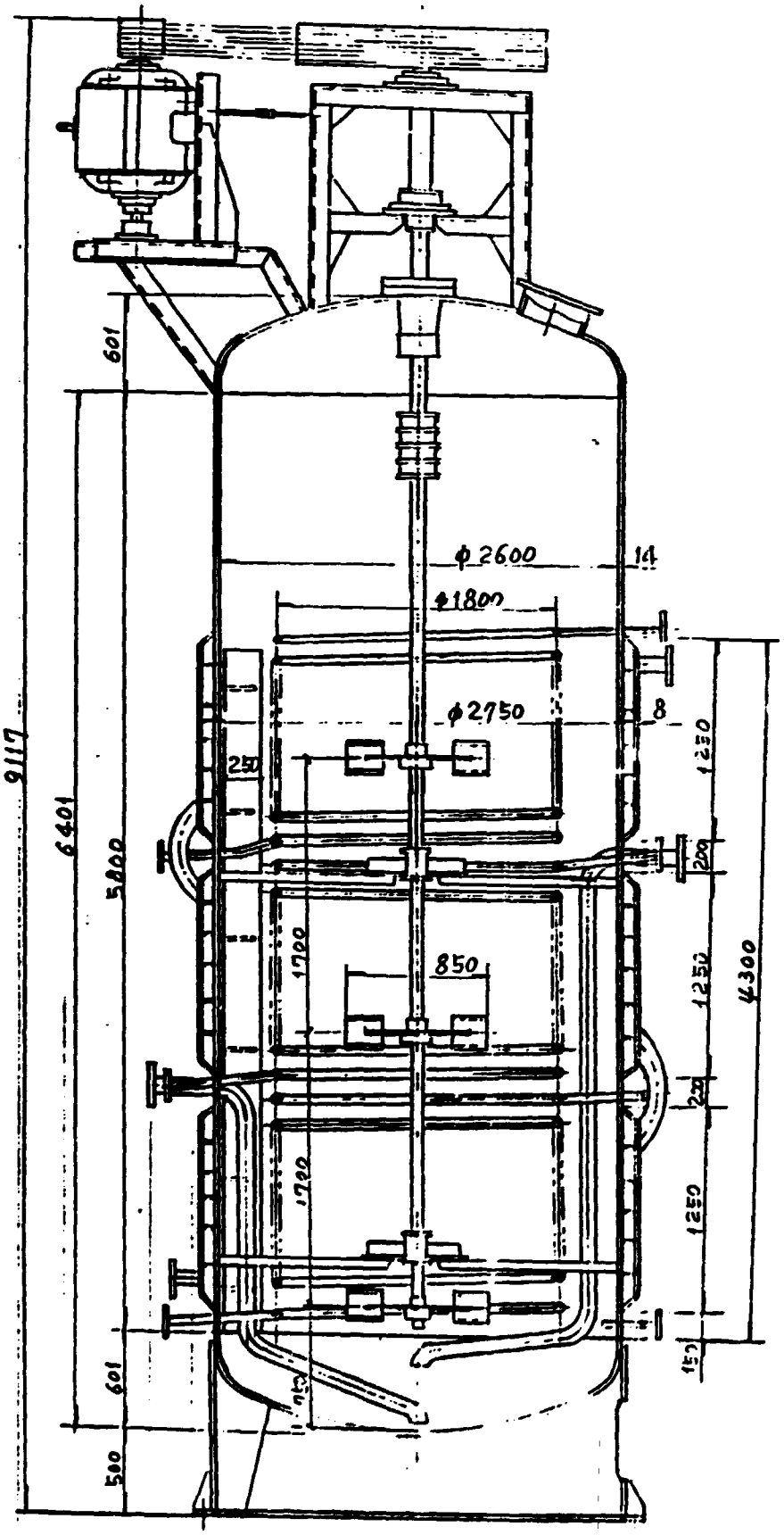
3.5. Design of vessels.

The 15 and 20M^3 fermenter are the same as in Penicillin.(2.5)
The 35M^3 fermenter has the following dimensions.

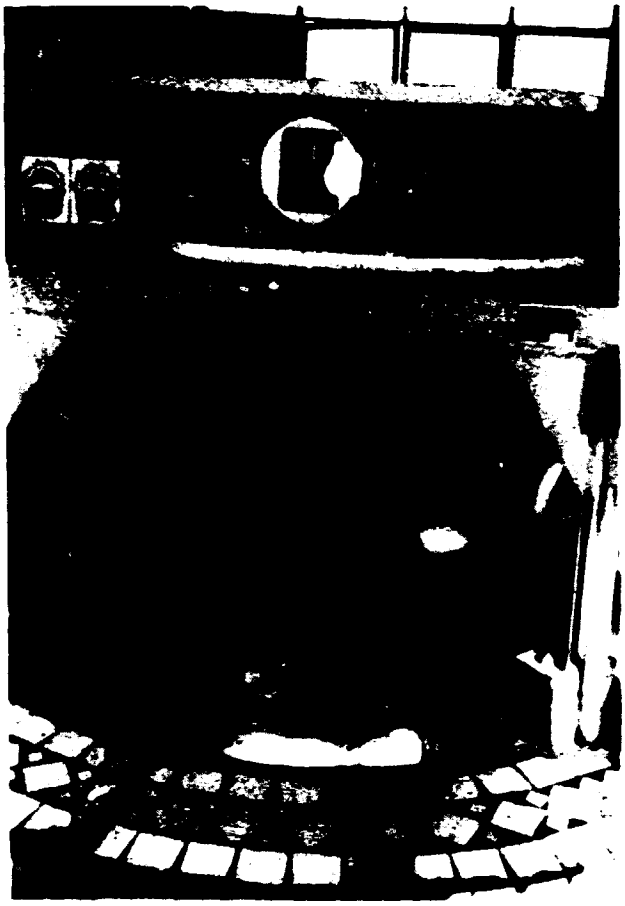
3.5.1. Vessel height .. 7000 mm
3.5.2. Vessel diameter .. 2600 mm
3.5.3. Agitator blade diameter .. 850 mm
3.5.4. Agitator shaft diameter .. 100 mm
3.5.5. Cooling coils from the bottom of the vessel .. 601 mm
3.5.6. Height of cooling coils .. 4300 mm
3.5.7. Totla volume .. 37000 lts
3.5.8. Working volume .. 22000lts
3.5.9. Metal thickness .. 14 mm
3.5.10. Agitator blades .. 210 mm/ 170 mm

D7

Fermenter



30 m³ 발효탱크



Fermenter

3.5.11. First blade from the bottom .. 750 mm

3.5.12. Liquid height .. 4150 mm

Air filtration is done the same way as in Penicillin.

3.6. Medium for the process and parameters.

3.6.1. Medium composition.

a) Soya bean flour .. 2.73% w/v

b) Corn starch .. 4 ,, ,,

c) Glucose .. 0.5 ,, ,,

d) Corn flour .. 2 ,, ,,

e) Sodium nitrate 0.8 ,, ,,

f) Zinc sulphate .. 0.011

g) Soya bean oil .. 0.2 ,, ,,

pH 6.0 initial, 8.5 final

3.6.2. Except glucose all the components are mixed in the mixing tank to $3M^3$ and pressed to the fermenter. The proceedure for sterilization is the same as in other fermenters.(2.6.2) Glucose is sterilized separately for 30 minutes at 115°C, cooled and added to the vessel. The $35M^3$ fermenter has both jacket and cooling coils. Water is pumped in to both of them for cooling and temperature control.

3.6.3. Seeded volume

	Medium	seed	additions	at harvest
15 M^3 ..	9.5 M^3	1.5 M^3	1.5M^3	11.5 M^3
20 M^3 ..	11.5 M^3	1.5 M^3	2 M^3	14 M^3
35 M^3 ..	17.5 M^3	4 M^3	2.5M^3	22 M^3

3.6.4. Parameters.

a) Temparature .. 28°C, through pneuma tic control with water supply to cooling coils. During summer water returned from Penicillin fermenters are used to circulate in the cooling coils here. No heating system is provided.

b) pH is measured and no automatic control system is available.

c) Agitation constant at 170 rpm.

d) Dissolved oxygen or oxygen uptake rate is neither measured nor controlled. Air is sparged as following:

For 10.5 M^3 .. 480 M^3/ hour

14 M^3 .. 600 ,, ,,

22 M^3 .. 800 ,, ,,

e) Assays are conducted once in 8 hours in shift till 96 hours.

3.6.5. Except water and oil additions are carried out.

3.7. Harvest.

After steaming the harvest line, the broth is taken directly to the retention tank, to be mixed with ion exchanger. No filteration is carried out.

3.8. Suggestions.

3.8.1. The method of culture preservation by lyophilization of spores may lead to long term deterioration of productivity. Previous experience with several hundred actinomycetous cultures have indicated severe loss of reproducibility and productivity. Preservation of vegitative mycelium grown in medium, distributed in vials 2 ml each and kept in liquid nitrogen, is the best method for actinomycetous cultures. Even preservation in glycerine and kept at low temparature can be tried. These have been suggested to them.

3.8.2. Checking of productivity should be carried out in two stage shaken flask fermentation, and not in single stage fermentation. It is advisable to prepare seed material by growing spores in shaken flask medium for 2-3 days, check pH, growth and P.C.V and transfer 5-8% seed to production flasks to check productivity.(3.1.4.)

3.8.3. Direct spores inoculation in the I stage inoculator should be avoided. The growth phase initially is prolonged for a large volume of 300 lts. It is advisable to use grown mycelium in flasks for 2-3 days, pool them and use 0.2-0.5 % seed for the inoculator tank. This will initiate good growth in the I stage itself. Also the inoculator will be susceptible for contamination due to lack of growth of actinomycetes in the initial stages.(3.2.6.)

3.8.4. There is only one autoclave for all the laboratories. This is also of primitive construction without even a temparature indicator, timer or exhaust cycle facilities. New autoclave has to be installed as preparation of seed will become critical if the medium and glass-ware are not sterilized properly.

3.8.5. As already indicated (2.8.1.) air filter modification or new filters are a must.

3.8.6. Air sparger modification should be done to improve growth and productivity,

3.8.7. New air flow controller recorder is necessary for proper aeration.

3.8.8. Agitator blades may be modified for proper oxygen distribution.
3.8.9. As the medium contains corn starch, corn flour and soya bean flour, it will definitely form a slurry when the volume of 3 M^3 only is mixed for 22M^3 seeded volume fermenter. There will be a tendency to form lumps and once they are heated it forms a hard crust. Heat cannot penetrate this lump and this may be one of the reasons leading to high rate of contamination at times. Enzyme hydrolysis of corn starch should be carried out for thinning out and better utilization of starch.
3.8.10. Glucose sterilization done separately is not properly sterilized. (3.6.2.) It is better to sterilize at 120°C for 20 minutes rather than improperly sterilizing at 115°C for 30 minutes. It is advisable to use pharmamedia and corn steep liquor instead of soya bean flour defatted. CSL along with calcium carbonate form a perfect buffer for the medium also.
3.8.11. As in the case of Penicillin fermenter open ended vent lines without any steam connection directly leading in to the dirty pit or tankwill harbour millions of bacteria. The system has to be modified with proper antifoam control and continuous oil additionby peristaltic pump or solenoid valve connection.
3.8.12. Acurate temperature indicator controllers are to be installed.
3.8.13. pH indicator controllers are very essential for the fermenters.
3.8.14. Analysis for pH, sugar, ammonia nitrogen should be done atleast 4 hourly and action should be taken against these on parameters. Batches record should be maintained on day to day basis with graphs in order to monitor the batches properly.
3.8.15. Production in plant.

a) - Average production in fermenter .. 4 gms/lt
   - Volume at harvest in 15M^3 fermenter .. 11 M^3
   - Yield per fermenter .. 44 Kg
   - No of batches taken in a year per fermenter .. 72
   - No of batches run for 2 fermenters.. 144
   - Total yield for 15M^3 fermenters .. 6336 Kg

b) - Volume at harvest in 20 M^3 fermenter .. 14 M^3
   - Yield per fermenter .. 56 Kg

- No of batches taken in a year per fermenter .. 72
- No of batches run for 2 fermenters .. 144
- Total yield for 20 M^3 fermenters .. 8064 Kg

c) - Volume at harvest in 35 M^3 fermenters. 22M^3
- Yield per fermenter .. 88 Kg
- No of batches taken in a year per fermenter .. 72
- Total yield for 35 M^3 fermenters .. 6336 Kg
- Total yield for 15M^3, 20M^3 and 35M^3 fermenters .. 20736 Kg
- Yield after purification at 58% .. 12026 Kg

4. Oxy tetracycline.

4.1. Culture and laboratory work.

4.1.1. The culture Actinomyces rimosus 7-746, produces on an average 8-9 gm/lt. The culture has probably come from China. There is a separate laboratory for slant preparation, culture maintenance and seed preparation. The colour of the slant is brownish, pigmentation if any is masked by the colour of the medium itself.

4.1.2. The slants are grown on the following agar medium:

a) Wheat brawn flour .. 7 %

b) Agar .. 2 %

pH 7.0

4.1.3. The slants are incubated at 37°C for 5-6 days, when they are ready. Strain selection is done on agar plates and checked continuously for proper production. Slants are usually utilized in a months time.

4.1.4. Culture preservation.

a) The surface growth/ spores are scraped and put in 2ml of sterile water and skim milk. 0.2 ml of this is suspended in ampules, freezed and hooked on to the lyophilizer for 18 hours, under 750 mm pressure. From one ampule usually 3-4 slants are prepared.

b) Spores are also kept on sterile soil. Soil is sterilized in flasks and dried under vacuum. Spores are then inoculated on this soil. This is stored for more than 6 months. From this slants are prepared and checked for productivity.

4.1.5. Culture testing in shaken flasks.

This is carried out in two stage fermentation in flasks. The following medium is used in I stage in flasks.

a) Corn starch .. 3%

b) Ammonium sulphate .. 0.4 ,,

c) Potassium di hydrogen phosphate .. 0.2 ,,

d) Corn steep liquor .. 0.5 ,,

e) Soya bean flour.. 0.3 ,,

f) Calcium carbonate .. 0,5 ,,

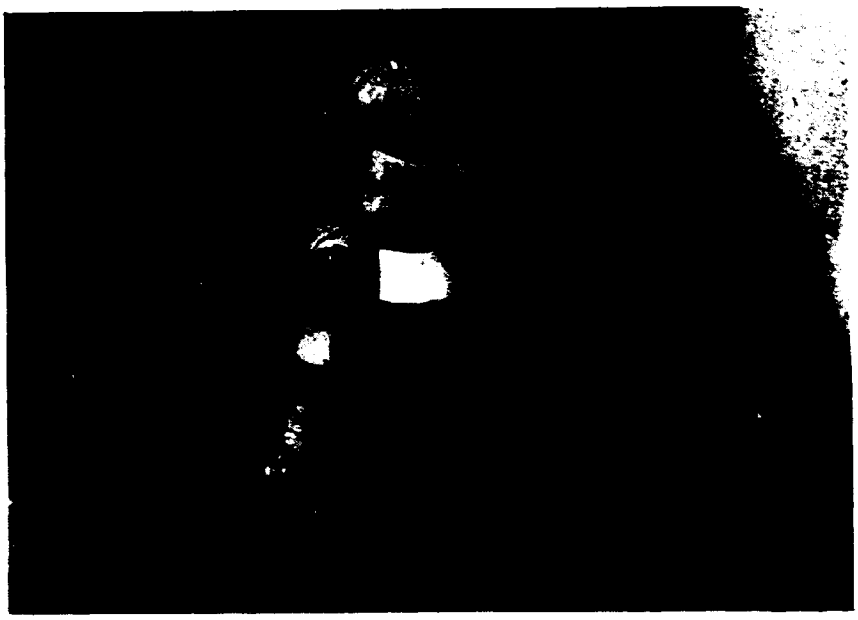
pH 6.6-6.8

One loopful of the culture is inoculated in this medium and run on shakers.

) Temparature .. 28-29°C

) Rpm .. 240

) 100ml/ 500 ml flask



OTC culture

Inoculator

Seed tank

iv) 30-32 hours age.

4.1.6. Culture testing in shaken flasks : fermentation flasks.

The following medium is utilized for the fermentation flasks.

a) Corn starch .. 7.5 %
b) Ammonium sulphate .. 0.75 ,,
c) Potassium di hydrogen phosphate .. 0.03
d) Soya bean flour .. 2.0 ,,
e) Calcium carbonate .. 0.8 ,,
f) Sodiun chloride .. 0.2 ,,
g) Soya bean oil .. 1.0 ,,

2.5 ml of this seed is used to inoculate 50 ml of fermentation flask medium.(5%). Parameters for the run.

i) Temparature ..28-29°C
ii) Rpm .. 240
iii) 50 ml/ 500 ml flask
iv) 168 hours age.

Final check is carried out in the central testing laboratory.

4.2. Inoculator tank.

4.2.1. There are 3 stainless steel 580 lt inoculator tanks. The design is same as found in Kanamycin.(3.2.1.) (2.2.1.)

4.2.2. Dimensions are the same as in Kanamycin.(3.2.2.)

4.2.3. Air filters,packing, sterilization are the same as in Penicillin or Kanamycin.(2.2.3.)

4.2.4. Media composition.

a) Corn starch .. 6 %
b) Soya bean flour .. 2.5 ,,
c) Sodium chloride .. 0.4 ,,
d) Ammonium sulphate .. 0.7 ,,
e) Calcium carbonate .. 0.1 ,,
f) Potassium di hydrogen phosphate .. 0.04 ,,
g) Soya bean oil .. 0.2 ,,

pH 6.5-6.8

4.2.5. Medium sterilization is done as in Penicillin.(2.2.5.)

4.2.6. From the slants the spores are inoculated in seed flasks as described in 4.1.5. After 30-32 hours of growth, three of these seed flasks are pooled and seeded in to the inoculator tank, 300 ml for 300 lt amounting to 0.1 % seed . This is done through the cap on the manhole cover with the help of an alcohol swab flame.

4.2.7. Parameters.

a) Temparature .. 28–29°C
b) Aeration .. 450 lpm
c) Agitation .. 220 rpm
d) Over pressure .. 0.2 Kg/cm^2
e) Time .. 48–72 hours

4.2.8. Only temparature control through hot water circulation in jacket is carried out. This is also done manually.

4.3. Seed tank.

4.3.1. There are 2 carbon steel 6 M^3 vessels and 1, 4 M^3 stainless steel vessel. The gereral vessel description is the same as in 2.3.1.

4.3.2. Vessel dimensions are the same as given in 3.3.2.

4.3.3. Air filters are the same including the packing material.

4.3.4. Medium composition.

a) Corn starch.. 6 %
b) Soya bean flour .. 2.5 ,,
c) Sodium chloride .. 0.4 ,,
d) Ammonium sulphate .. 0.7 ,,
e) Calcium carbonate .. 0.6 ,,
f) Potassium di hydrogen phosphate .. 0.04 ,,
g) Soya bean oil .. 0.1 ,,

pH 6.5–6.8

4.3.5. Medium sterilization is carried out as in other antibiotics. (2.3.5.)

4.3.6. Seeding is done through the inoculum transfer line. 300 lts seed are transfered to make 2 M^3 seeded volume. For the 30 M^3 fermenter 3 M^3 seeded volume is taken up as a special case. Medium analysis is done for :

a) Sugar
b) Ammonia nitrogen
c) Phosphorous
d) Ferric oxide

4.3.7. Parameters.

a) Temparature .. 29°C
b) Aeration .. 3000 lpm
c) Agitation rpm .. 220
d) Over pressure .. 0.2 Kg/cm^2
e) Running time .. 36 hours

4.3.8. Temparature and aeration control are manual.

4.4. Fermenters.

There are 6 fermenters of 15M^3 and 1 of 35M^3 carbon steel make. The description is the same as in other products.

4.5 Design of vessels.

Design and dimensions of 15 M^3 are as in 2.5. and the 35 M^3 are as in 3.5.

4.6. Medium for the process and parameters.

4.6.1. Medium composition.

a) Corn starch .. 3.5 %
b) Corn flour .. 3.5 ,,
c) Soya bean flour .. 1.5 ,,
d) Sodium chloride .. 0.2 ,,
e) Ammonium sulphate .. 0.7 ,,
f) Calcium carbonate .. 0.7 ,,
g) Potassium di hydrogen phosphate .. 0.03 ,,
h) Soya bean oil .. 0.1 ,,

pH 6.0-6.3

4.6.2. All the components of the medium are mixed in the mixing tank and sent under pressure to the fermenter. It is stefilized in the same manner as in the other fermenters.

4.6.3. Seeded volume.

15 M^3 .. 9 M^3 + additions 2M^3 .. at harvest .. 10.5 M^3
35 M^3 .. 20M^3 + ,, 4M^3 .. ,, .. 22 M^3

4.6.4. Parameters.

a) Temparature .. 29°C , with pneumatic control valve with ordinary water supply to the cooling coils.

b) Aeration .. 1: 0.7

c) Agitation .. 170 rpm

d) Assay done once in 8 hours till harvest at 168-200 hours age.

4.6.5. Addition during fermentation.

a) There is 6 M^3 corn starch solution tank. 30% corn starch is prepared, liquified with α amylase addition. The temparature of the starch solution is kept at 40°C all the time. 4 shots are added after 24 hours age, every 20 hours one shot of 250 lts. There is one 500 lts stainless steel holding tank with crude level indicator.

b) There is a liquid ammonia tank of 50 lt capacity. 25% ammonia

Ammonia tank

is kept without sterilization and added to the fermenter from 50-140 hours to keep pH under control.

c) Soya bean oil additions are donefrom time to time to manually control foaming.

4.7. Harvest.

The broth is taken to the pre treatment tank and pH is adjusted with oxalic acid to pH 1.6, before filtration.

4.8. Suggestions.

4.8.1.Culture preservation by lyophilization, as already indicated in (3.8.1.) may lead to loss of viable spores and productivity. Using sterile soil may be an old practice, but one has to be very careful in the proceedure of sterilization and maintenance. The best method suggested, as earlier is to preserve vegetative mycelia in liquid nitrogen.

4.8.2. Testing of culture in shaken flasks.

The medium containing 7.5% corn starch should be enzyme hydrolysed and then used. Direct starch sterilization will definitely lead to thicker non sterile medium and sometimes prolongation of fermentation cycle. For such a rich medium shaker rpm appears very low. The oxygen transfer in such a medium in initial stages will be low. It is better to use high rpm shakers upto 400 rpm for better productivity.

4.8.3. Seeding % in inoculator tank as seen (4.2.6.) is 0.1%. Though it is claimed that this is ideal it is better to check and increase it to 0.3-0.5%. This will enhance the seed growth prior to transfer at 48-72 hours age.

4.8.4. Air filter modification or new cartridge filters must be carried out/ installed.

4.8.5. Air sparger modification along with agitator blade will lead to better oxygen absorption.

4.8.6. New flow recorder controllers are necessary for better aeration control.

4.8.7. Vent line modification has to be carried out as specified in (3.8.11.)

4.8.8. Acurate temperature indicator controllers are needed.

4.8.9. pH indicator and controller with peristaltic pump or solenoid valves and addition pots have to be provided for proper pH control.

4.8.10. a) The fermentation medium contains corn starch 3.5% along with corn flour 3.5% and soya bean flour 1.5%. Proper mixing tanks are not there as the volume of mixing tank, $/m^3$ is very low. At least

6 M^3 mixing tank is essential especially for a working volume of 22M^3 in the 35 M^3 fermenter.

b) Due to the composition, the medium is very thick and viscous hence sterilization is difficult. It is worth while to hydrolyse the corn starch and then mixup the rest of the ingredients, so that the medium is not very thick and the organism is able to utilize the starch readily.

c) Corn steep liquor and pharmamedia must be checked in the medium for productivity.

4.8.11. Additions to the fermentation cycle.

a) Starch addition of 75 Kg, 4 times at the rate of 0.75% of the volume of the fermenter is done at random. Broth analysis carried out at critical stages 2Or 4 hourly will give correct indication of starch availability.

b) There is no readily available sugar source like glucose in the medium, till the time starch or corn flour is degraded in to assimilable sugar. Initial addition of 0.5% or 1% glucose has to be checked for the culture, whether there is repression or normal growth and production. This can be carried out in the pilot plant trials and steps can be taken accordingly.

c) Liquid ammonia 25% is added without any filtration. Bacterial filtration is a must for any addition of liquid without sterilization in to the fermenter. The whole tank can be sterilized empty and ammonia can be pressurized through Zeits filter in to the tank. The lines should be sterilized with steam and connected properly, and not with air.

4.8.12. Production in plant.

a)- Average production in fermenter .. 10 gm/lt
- Volume at harvest in 15 M^3 fermenter .. 10.5 M^3
- Yield per fermenter .. 105 Kg
- No of batches taken in a year/ fermenter .. 39
- Total no of batchesrun for 6 fermenter .. 234
- Total yield for 15 M^3 fermenter .. 24570 Kg

b)- Volume at harvest in 35 M^3 fermenter .. 22 M^3

- Yield / fermenter .. 220 Kg
- Total no of batches taken for fermenter .. 39
- Total yield for 35 M^3 fermenter .. .. 8580 Kg
- Total yield for 15 M^3 and 35 M^3 fermenters ..33150 Kg
- Yield after purification at 61% ..20221 Kg

5. Rifampicin.

5.1. Culture and laboratory work.

5.1.1. The culture streptomyces mediterraneous -80, produces on an average 5-6 gm/lt. There is a seperate laboratory for the preparation maintanance and seed preparation. The culture is light brown surface culture on slants with faint yellow pigmentation on the agar. Colonies on the agar look light brownish red with 5 mm diameter growth of rosettes.

5.1.2. The culture slants are grown on the following agar medium.

a) Beef extact .. 0.1 %
b) Casein hydrolysate .. 0.2 ,,
c) Yeast powder .. 0.1 ,,
d) Glucose .. 1.1 ,,
e) Agar .. 2.0 ,,
pH 7.2

5.1.3. The slants are incubated at 27°C for 5-7 days and then in the refrigerator for 2 months at 4°C. It is claimed that upto 3 months storage there is no loss in production. Strain selection is carried out regularly on agar plates and then on to slants. Testing is done regularly from the slants.

5.1.4. Continuous sub culturing and strain selection are done and no permanent preservation is carried out.

5.1.5. Culture testing in shaken flasks.

This is carried out in 2 stage fermentation. The following medium is used in I stage flask.

a) Glucose .. 2.2%
b) Soya bean flour .. 1.0 ,,
c) Casein hydrolysate .. 7 mg%
d) Sodium chloride .. 0.5%
pH 7.5

One loopful of the culture in inoculated in to this medium and run on shaker.

i) Temparature .. 27°C
ii) Rpm .. 240
iii) 50 mlin 500 ml flas.
iv) Age .. 72 hours

5.1.6. Culture testing in fermentation flasks.

Thefollowing medium is used in fermentation flasks.

a) Glucose .. 9.35 %
b) Soya bean flour .. 3 ,,
c) Ammonium sulphate .. 0.9 ,,
d) Potassium di hydrogen phosphate .. 0.17 ,,
e) Propyleneglycol .. 0.5 ,,
f) Magnesium sulphate.. 0.01 ,,
g) Glycerine .. 4.0 ,,
h) Calcium carbonate .. 0.9 ,,
i) Sodium barbiturate .. 0.17 ,,
j) Copper sulphate .. 0.0033 ,,
k) Ferrous sulphate .. 0.01 ,,
l) Zinc sulphate.. 0.05 ,,
m) Cobalt chloride .. 0.0002 ,,
n) Manganous sulphate .. 0.0001 ,,
o) Ammonium molybdate .. 0.0001 ,,
pH 7.8

5 ml of seed (10%) is used to inoculate 50 ml of the flask medium.
Parameters are

i) Temparature .. 27°C
ii) Rpm 240
iii) 50 ml in 500 ml flasks
iv) Age 240 hours

Final check on activity is done in the central testing laboratory.

5.2. Inoculator tank.

5.2.1. There are 3 stainless steel tanks of 500 lt capacity. The transfer line is slightly different as it is punched at the sides of the tank. Rest of the connections are the same as in Kanamycin. There is a ring sparger unlike in other inoculators.

5.2.2. Dimensions are the same as in Kanamycin. (3.2.2.)

5.2.3. The air filter jacket is connected with steam unlike in other inoculators.

5.2.4. Medium composition.

a) Soya bean flour .. 1.2 %
b) Glucose .. 3.4 ,,
c) Potassium di hydrogen phosphate .. 0.1 ,,
d) Ammonium sulphate .. 0.6 ,,
e) Calcium carbonate .. 0.5 ,,

Inoculator

Inoculator and seed tank

f) Magnesium sulphate .. 0.1 %
g) Manganous sulphate .. 0.001 ,,
h) Zinc sulphate .. 0.005 ,,
i) Ferrous sulphate.. 0.001 ,,
j) Soya bean oil .. 0.2 ,,
pH 6.5

5.2.5. Medium sterilization is done in the same way (2.2.5.)

5.2.6. From the slant one loopful of spores are inoculated in to the seed flasks as in 5.1.5. After 72 hours the growth and pH are checked and 5 ml of this is transfered to II seed flasks of 100 ml in 500 ml flasks. This is run in the same manner as shown at 5.1.5 ito iv for 48 hours. Ten of these flasks are pooled (1 lt seed) to inoculate 150 lts medium in the inoculator tank.

5.2.7. Parameters.

a) Temparature .. 27.5°C. The control here is done automatically with pneumatic control valve.

b) Initial aeration is kept at 1:0.5 (75 lpm)upto 36 hours and increased to 1:1.2 (180 lpm) till it is transfered.

c) agitation .. 220 rpm
d) Over pressure .. 0.2 Kg/cm^2
e) Time .. 53-68 hours

5.2.8. Except for the temparature automatic control no other controls are available.

5.3. Seed tank.

5.3.1. There are 3 carbon steel tanks of 3 M^3 capacity. The air inlet is from the side of the tank. Steam condensate from the seed transfer line is drained through inoculum valve in transfer line.

5.3.2. Vessel dimensions are the same as given in 2.3.2.

5.3.3. Air filter is the same as in other seed tanks. 2.3.3.

5.3.4. Medium composition.

a) Soya bean flour .. 1.2 %
b) Glucose .. 3.4 ,,
c) Potassium di hydrogen phosphate .. 0.05 ,,
d) Ammonium sulphate .. 0.6 ,,
e) Calcium carbonate .. 0.5 ,,
f) Magnesium sulphate .. 0.1 ,,
g) Manganous sulphate .. 0.001 ,,
h) Zinc sulphate .. 0.005 ,,
i) Ferrous sulphate .. 0.001 ,,

j) Soya bean oil .. 0.2 %
pH 6.5

5.3.5. Medium sterilization is carried out like in other seed tanks. (2.3.5. or 4.3.5.)

5.3.6. Seeding is done through inoculum transfer line, to have 1.5 M^3 seeded volume.

5.3.7. Parameters.

a) Temparature .. 27.5°C
b) Aeration initially 1:.5, later on increased to 1:1.2
c) Agitation rpm 220
d) Over pressure ..0.2 Kg/cm^2
e) Time 36-48 hours.

5.3.8. Temparature controlled and no other controls are provided.

5.4. Fermenter.

There are 4, 20 M^3 carbon steel fermenters. Description is the same as in Penicillin.(2.4)

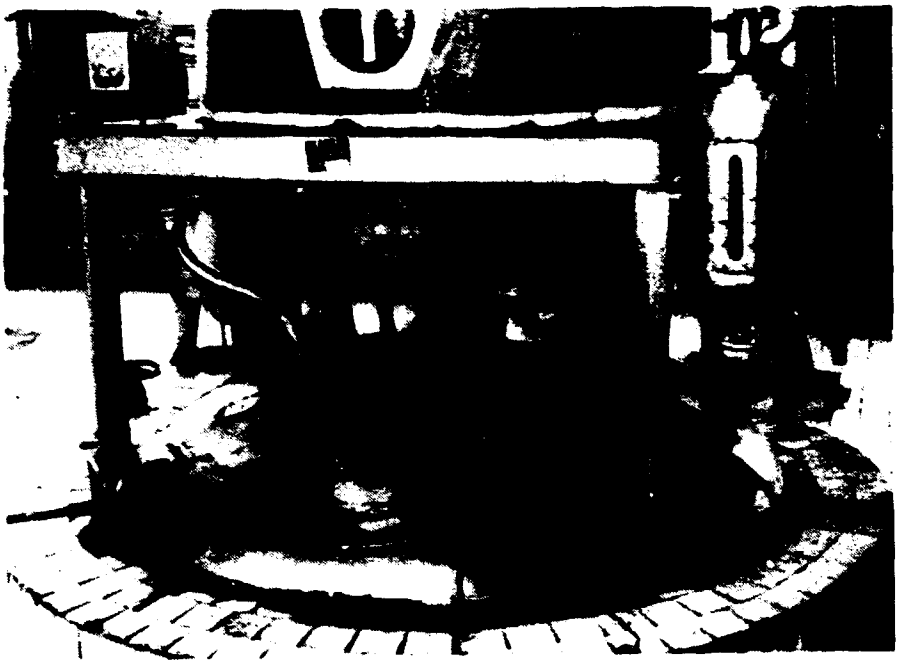
5.5. Design of vessels.

Design and dimensions are the same as in 2.5.

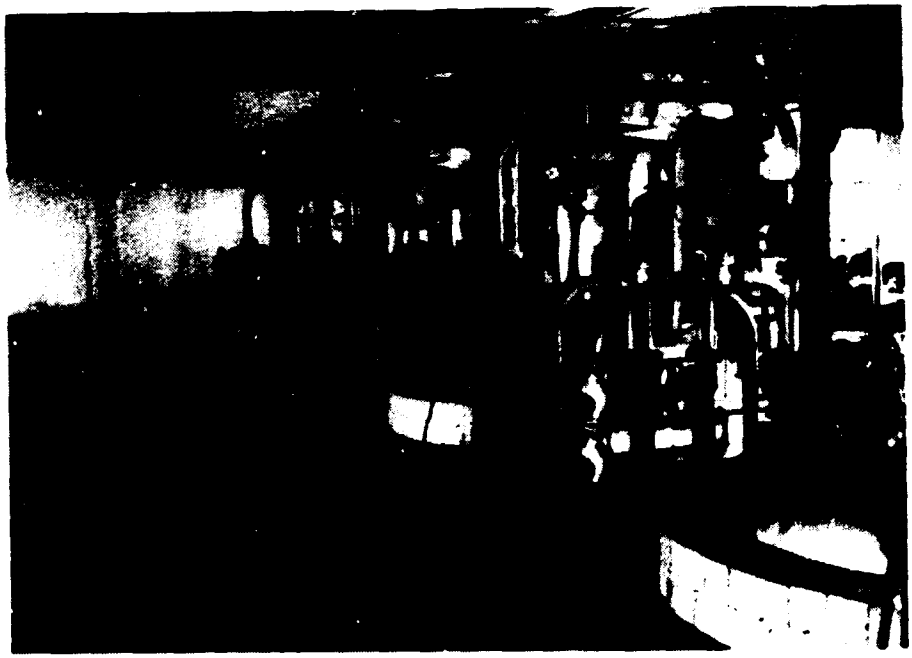
5.6. Medium for the process and parameters.

5.6.1. Medium composition .

a) Soya bean flour .. 3.5 %
b) Glucose .. 6.5 ,,
c) Potassium di hydrogen phosphate .. 0.05 ,,
d) Cane sugar .. 7.0 ,,
e) Ammonium sulphate .. 1.0 ,,
f) Calcium carbonate .. 0.95 ,,
g) Magnesium sulphate .. 0.085 ,,
h) Manganous sulphate .. 0.0004 ,,
i) Zinc sulphate .. 0.005 ,,
j) Ferrous sulphate .. 0.001 ,,
k) Copper sulphate .. 0.00033 ,,
l) Ammonium molybdate .. 0.0001 ,,
m) Sodium barbiturate .. 0.8 ,,
n) Propyleneglycol .. 0.5 ,,
O) Silicone oil .. 0.025 ,,
p) Soya bean oil .. 0.1 ,,
pH 6.5



Fermenter



5.6.2. The medium is mixed in the mixing tank and sent up to the vessel, sterilization follows in the same manner.

5.6.3. Seeded volume of the fermenter is 14 M^3.

5.6.4. Parameters.

a) Temparature .. 27.5°C. Control is manual unlike in other antibiotics.

b) Aeration .. upto 12 hours 1:0.1, then gradually increased to to 1:1.

c) Agitation .. 170 rpm

d) Age .. 180–240 hours

5.6.5. Additions during the cycle.

a) Oil addition done according to foaming conditions. About 330 lts are consumed.

b) 30% Sodium hydroxide solution is kept in a tank under pressure. One stainless steel line is connected to the fermenters from this tank. This line is connected to the air inlet line. As and when pH control is required this is added.

c) 800 lts of the original composition of the medium is added 3 times.

5.7. Harvest.

The broth is taken to the pretreatment tank for cooling and for filtration.

5.8. Suggestions.

5.8.1. Culture preservation as advised in 3.8.1. is to be carried out preferably in liquid nitrogen.

5.8.2. Testing of culture in shaken flasks.

The fermentation flask medium contains 9.35% glucose. If the whole of it is taken in the initial medium, there is bound to be catabolic repression due to excess of glucose. The pH will tend to fall very rapidly upto 20–36 hours age as the medium also contains Potassium di hydrogen phosphate o.17%. It is advisable to take only 2% glucose initially and add the rest of the glucose and propyleneglycol every 24 hours in 6 or 7 additions, after sugar analysis.

5.8.3.Air filter modifications as suggested earlier (2.8.1.) is a necessity.

5.8.4. Ring sparger along with agitator blade modification will lead to better productivity.

5.8.5. New air flow controller and recorder are necessary for proper

aeration and control.

5.8.6. Vent line modification should be carried to avoid contamination.

5.8.7. Temparature controllers should be made automatic with electronic indicator controllers.

5.8.8. pH indicator and controllers are very essential for the prolonged fermentation cycle.

5.8.9. a) The initial medium in fermenter contain 7% cane sugar and 6.5% glucose. This will lead to catabolic repression as indicated in 5.8.2. Initial 2% glucose with 0.5% cane sugar may be the ideal medium. After 30-36 hours, after checking the left over sugar, the rest of the glucose, and cane sugar can be added in stages. Sodium barbiturate and propyleneglycol also should be added in stages. This will give proper sugar feed to the fermenter and also the precursors at critical time.

5.8.10. Addition of sodium hydroxide through the fermenter air line should be discontinued. There are always chances of the caustic soda remaining in the line after the batch, and pH abnormalities will occur in the next batch. Caustic soda will also corrode the ms pipe line leading to pitting and pores. The line may have to be replaced subsequently.

5.8.11. Production at plant.

- Average production in fermenter .. 3.5 gm/lt.
- Volume at harvest in 20 M^3 fermenter .. 11 M^3
- Yield per fermenter .. 38.5 Kg
- No of batches taken in an year/ fermenter .. 21
- NO of batches run for 4 fermenters .. 84
- Total yield in fermenters .. 3234 Kg

  Yield after purification at 25% .. 808.5Kg

6. Support activities.

6.1. Pilot plant.

6.1.1. There are 2 inoculators of 200 lts each. These are made of stainless steel and the design is the same as seen in production block. The inoculum developed is utilized to seeding the seed tank.

6.1.2. There is one, $1M^3$ seed tank of same design as in production block. The II stage seed is utilized to seed the fermenter.

6.1.3. There are two carbon steel 3 M^3 fermenters with a working volume of 1.8 M^3. This is a jacketed fermenter with inoculum inlet line, air inlet line, sample line, harvest line with respective steam connections.

a) Temparature control is done manually.

b) Air flow is also controlled with manual operation with recorder to indicate the aeration rate.

6.1.4. It is very essential that in order to study the culture characteristics of new products and to do scale up work the following accessories are to be procured, and installed.

a) Acurate temperature indicator, controller and recorders.

b) pH indicator, controller and recorders.

c) D O_2 indicator, controller and recorcers.

d) P CO_2 indicator, controller and recorders.

e) Cartridge air filters

f) Vessel has to be clad inside with 2 mm thick stainless steel 316 quality sheet.

g) Sparger and agitator blades are to be made of SS 316, and modified as per the dimensions given to them.

h) Different media components are to be made available to run the pilot plant.

i) Stainless steel addition pots are to be installed for dozed additions during the fermentation.

6.2. Research and development.

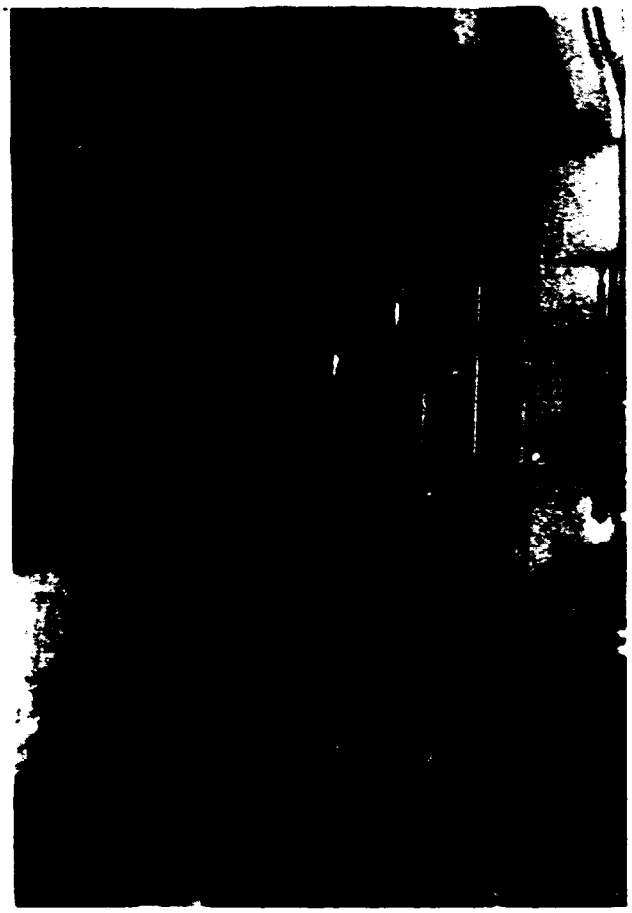
6.2.1. Microbiology.

This R&D group is headed by Mrs.Le Ke Suk trained in U.S.S.R. The usual methodology is to do strain improvement, media studies, temparature studies with the limited equipment available.No work is carried out on Penicillin strain. Some times due to non availability of pilot plant the strains are tried directly in production fermenters.

a) In the case of Rifampicin, by the method of strain selection

Pilot plant - Inoculator

Seed tank



R&D purification

and media improvement from 3000 u/ml the production rate has gone up to 5000 u/ml.

b) Strain selection and media work is also carried out for Tetracycline which is at present at 10 gm/lt.

c) Erythromycin strain is also being worked out. At present the yields are low at 2 gm/lt.

d) Gentamicin is also being worked in the pilot plant level with very low yields.

Lack of different raw materials that are easily available in other countries like cotton seed protein flour, c.s.l, hydrol are a handicap; Lack of equipment like shakers with different speed and incubator shaker,impedes any kind of research work.

6.2.2. Attached to the R&D, there is a laboratory for checking on purification methods. They have different glass columns of 8-10 cm diameter and minimal amounts of different resinsto check for different purification processes. They do not have any Medium pressure liquid chromatography or droplet counter current chromatography or laboratory counter current extractor.

7. Services.

7.1. Compressed air.

7.1.1. There are 13 pistontype compressors of 10M^3/ minute and 3 compressors of 40 M^3/ minute. The smaller one has 75 KW motor and the bigger one 250 KW motor, AC. Air is supplied to all the divisions from here.

7.1.2. At the outlet of the compressor the temparature of the air is 120°C. There are different kinds of air receivers . 3 of 5M^3, 5 of 1.5M^3 and 4 of 4 M^3 capacity. They are on both sides of the compressor house. After the receiver there are water coolers where the pipe lines of water are vertically mounted inside the vessel. Here as much of the moisture is removed from the air. The water return line and air purging is below the groundlevel.

7.1.3. After the water cooler there is an oil separator with pyramidal plates on top of one another. There are several holes on the plates. This whole structure can be dismantled and taken out for cleaning. Oil from the air is supposed to be removed to an extent inthis separator.

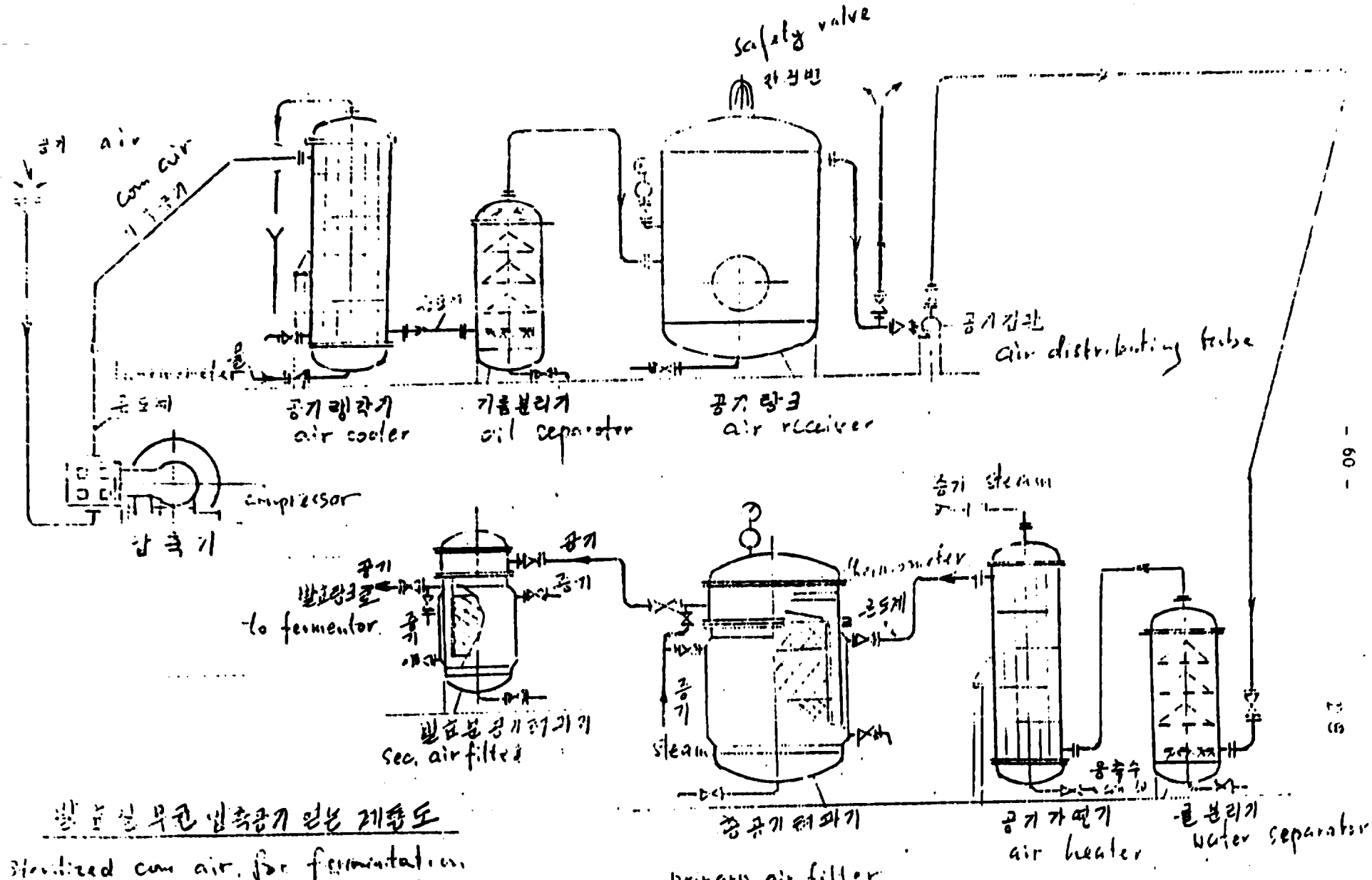
7.1.4. By the time air enters the fermentation block the trmparature is 35°C. During winter time when the ambient temparature is very low, air is heated to 35°C, with steam in jacket. During the rainy season the humidity is high (not measured) and there is quite a lot of moisture in the air. The contamination rate is high as the air filters are inefficient during this period. Air is heated to 50°C to keep the moisture in vapour state.

7.1.5. The two parallel lines on both sides of the compressor house merge and feed the fermentation and other blocks. For each antibiotic there are two primary filters inside the building. From this air is branched off and lines are given to individual vessels. Each vessel as it has been explained already has individual filters.

7.1.6. Suggestions.

a) Total air requirement for the fermentation block with 1:1 aeration works out to 350 M^3/ min. Over all requirement is claimed at 430 M^3/ min. The present over all capacity is 210 M^3/ min, if all of them are running . Hence the air line pressure is low and not all the fermenters are in commission. Two turbo compressors or centrifugal ones of 200 M^3/min will solve the air problem to a great extent.

Complete Air system



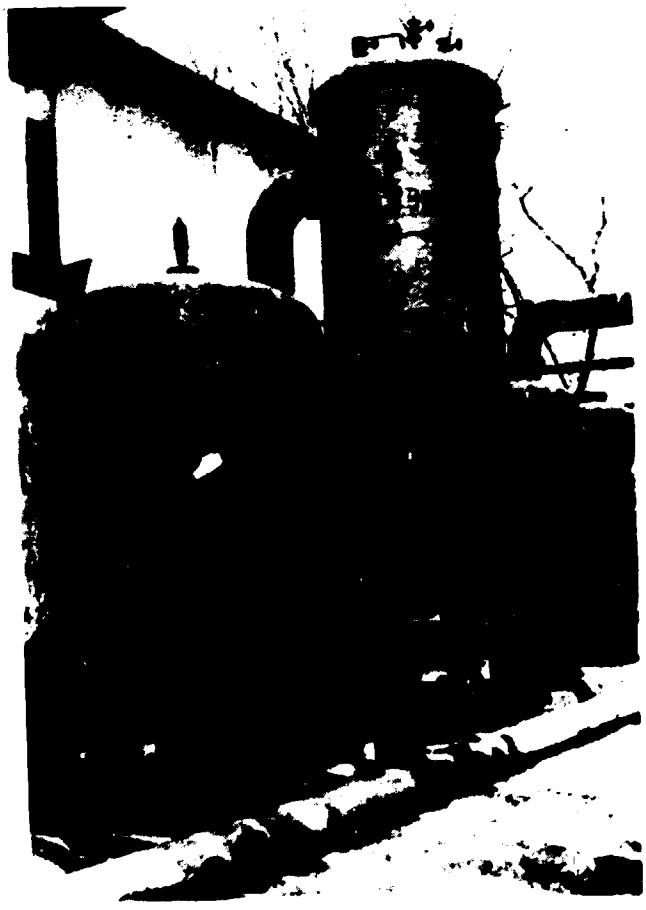
발효용무균압축공기 만듦 계통도

Sterilized com air for fermentation

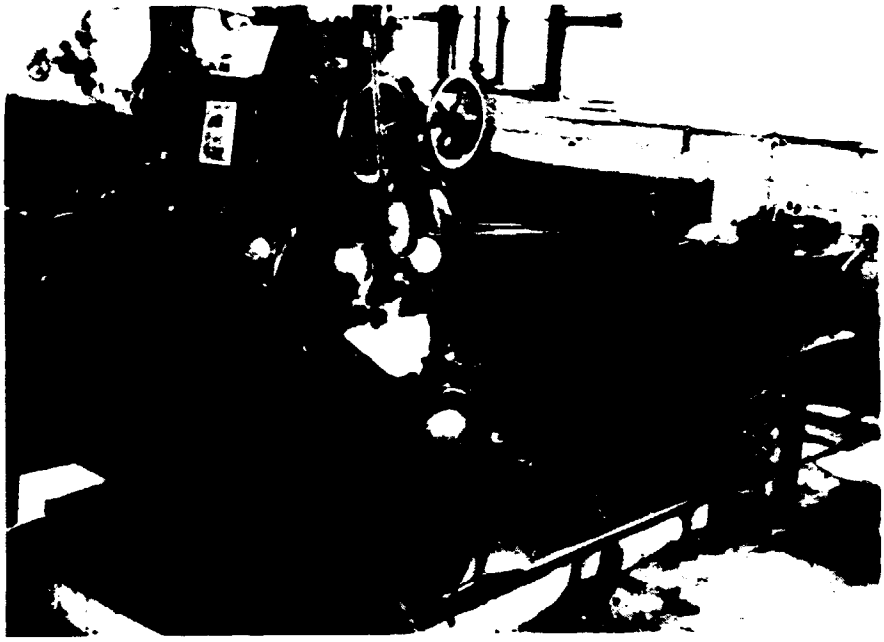
Air compressors

Air receivers

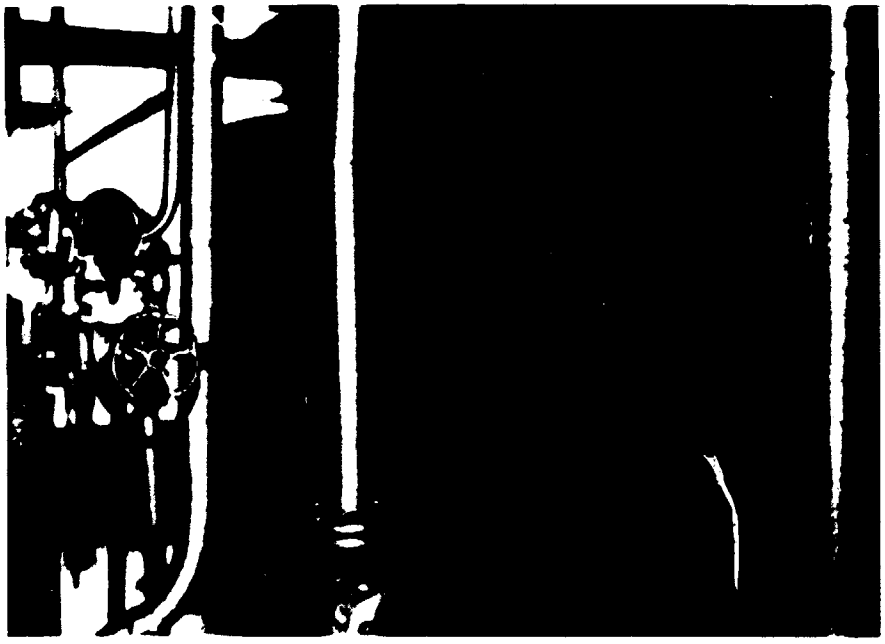
Air compressor



Moisture separator



Ammonia compressor



Primary air filters .

b) Efficient system of moisture removal is to supply brine to the cooling coils and thus reduce the air temparature to below dew point. As the water tamparature is 14°C during summer this cannot be achieved.

c) It is better to get rid of several number of receivers of smaller capacity and build one 70m^3 capacity air receiver for the backup supply in case of power break down. As the small capacity receivers are all parallel in line, back up capacity will be very low.

d) As already indicated absolute cartridge filters with their pre filters are very essential.

7.2. Steam.

7.2.1. All the boilers are coal fired boilers. The coal mine is 12 KM from the factory site. The average consumption is 60 ton/ day in summer and 80 ton/ day in winter The following are the boiler capacity.

a) 10 ton/ hour .. 2 Nos.
b) 8 ,, ,, .. 1 ,,
c) 5 ,, ,, .. 1 ,,
d) 2.5 ,, ,, ,, 2 ,,
e) 2.3 ,, ,, .. 1 ,,
total 40.3 ton/ hour

7.2.2. The boiler house serves for the whole plant. For antibiotics approximately 27 tons/ hour is utilized and the rest is for other products. The main line pressure for antibiotics is maintained at 4 Kg/cm^2. Water is treated with sodium chloride to remove matallic ions. The precipitated salts are removed in a tank.

7.2.3. Suggestions.

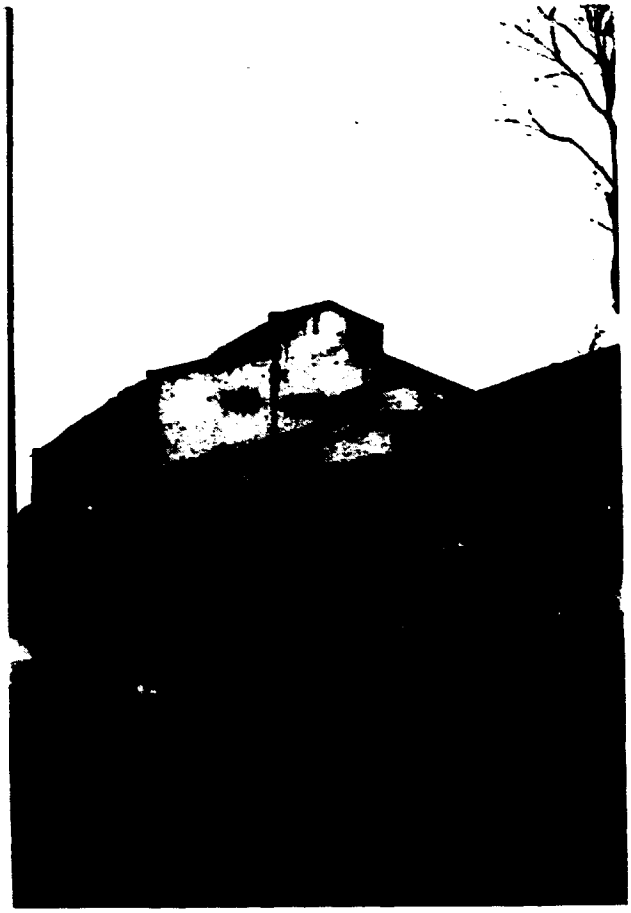
a) Diesel oil availability seems to be very limited. It has to be imported for running oil fired boilers. It is worth while to put 2 oilfired boilers of 20 tons/ hour each, which are very compact in size and does not pollute.

b) The present boilers may pose a health hazard to personnel because of heavy coal dust. The place also requires cleaning all the while.

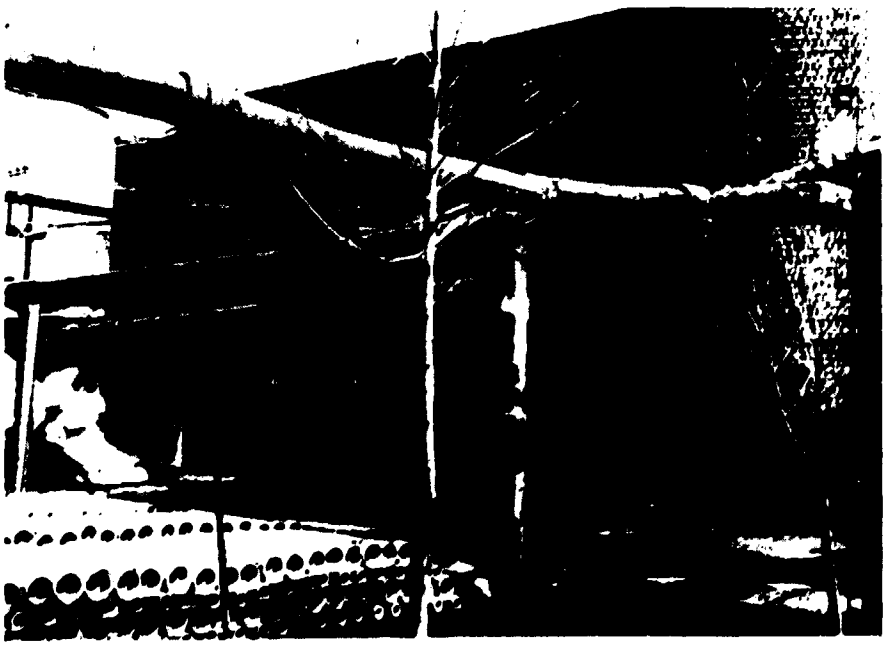
c) Water treatment plant is essential to soften up the water for the boiler. This can be procured as a package deal along with the boiler.

7.3. Water.

7.3.1. The main water supply is from the Daedongong river, which is



Boiler house



one kilometer away. There is constant water supply and no heavy flooding during the rainy season. The river water is taken to two concrete wells from where this is pumped to the plant. There is a pump house a little above the river level and this is approachable by a narrow unpaved road.

There is bore well inside the factory premises which gives supply at 40 M^3/ hour.

7.3.2. For cooling water there are two pumps which supply at the rate of 430 M^3/ hour and 180 M^3/ hour respectively. For industrial water there are four pumps which supply at the rate of 340 M^3/ hour. The total industrial water consumption per day is 18000 M^3.

7.3.3. No treatment is given to the water which is supplied to the plant, to remove the matallic ions.

7.3.4. Suggestions.

a) The pumps as like other equipment are outdated. New centrifugal pumps are required with a rated capacity of 500 M^3/ hour which can give uninterrupted supply to the plant.

b) The water must be treated with alum, sand filtered, and taken through ion exchange for theuse in fermenters for media mixing etc. This de mineralised water will be the best as there is already metallic contamination in the water to add up to the carbon steel fermenters. River water can be taken directly for cooling coils and the jacket of vessels.

7.4. Coolant system.

7.4.1. There are 9 ammonia compressors one of 40,000 K cal/hour and the rest with 10,000 K cal/ hour. As the ambient temparature is very low for atleast 6 months ina year, coolant is not a problem in the plant. This is quite different from the situation in Asia, where enormous amount of money will be spent for closed cooling water system. Methanol is utilized for cooling coils. Most of the cooling is utilized in the down stream processing area.

7.5. Electricity.

7.5.1. Sub station for the plant is located just outside the factory area manned by the personnel concerning that division.

7.5.2. Power is distributed as under.

a) Penicillin .. 560 KVA

b) Kanamycin .. 235 KVA

c) OTC .. 1000 KVA

Sub station

Control panel

d) Rifampicin .. 100 KVA
e) Water supply .. 20 KVA
f) Sewage .. 100 KVA
g) Heating .. 560 KVA
h) Workshop .. 200 KVA
i) Packing .. 150 KVA
j) Glass injectible .. 300 KVA
k) Line laying .. 200 KVA

7.5.3. Suggestions.

Supply lines and distribution boards are very old and need replacement. Many of the boards in corridors are open and within reach of any staff. Remodelling of the electrical layout is very essential as it is a safety hazard.

7.6. Civil.

7.6.1. The building area for fermentation works out to approximately 10,885M^2 with the following break up.

a) Penicillin ..1850 M^2
b) Kanamycin ...1134 ,,
c) OTC ..1440 ,,
d) Rifampicin ..1520 ,,,

Total	.. 5944 M^2
e) Packing	.. 1179 ,,
f) Pilot plant and others	.. 732 ,,
g) Change rooms, corridors, stairs, depot	.. 3030 ,,
Total	10885 M^2

7.6.2. As refered in 8.1.5. & 8.1.6. the buildings many of them need repair and painting. Road conditions are to be improved upon.

8. Maintenance.

8.1. General.

8.1.1. There is a main manufacturing workshop, where most of the material required for the plant are made. Vessels, air filters, covers, filter press, pulleys nuts and bolts are all manufactured . They have 22 machines in the workshop area. They are mostly machine tools, boring lathes, grinding, hydrolic press etc. Plate bending machine, cast iron facility are also available. Parts manufactured here are sent to different departments for their use. Other than this there are small scale maintenance team in every section.

8.1.2. There are totally 110 workers in the maintenance group. Of which 75 are in the manufacturing workshop and 35 in the maintenance area. Out of the second group 20 are in shifts during the night operations. In the antibiotic division all maintenance operations are carried out by the work team consisting of 25 persons.

8.1.3. The electrical maintenance team consist of 27 employees. 5 more persons work in day or day/night shifts.

8.1.4. Instrumentation work is carried out by 4 persons in day/ night shift.

8.1.5. Civil maintenance is carried out by a work team of 15 persons. They carry out repairs to buildings, drainage , campus roads, and wate r supply.

8.1.6. a) Due to lack of funds, many of the buildings are in dilapitated and unpainted conditions. Roads need to be improved upon, open manholes are to be covered.

b) Quality ss valves and better gaskets are needed for the vessels

c) Electrical installations and wiring work need replacement.

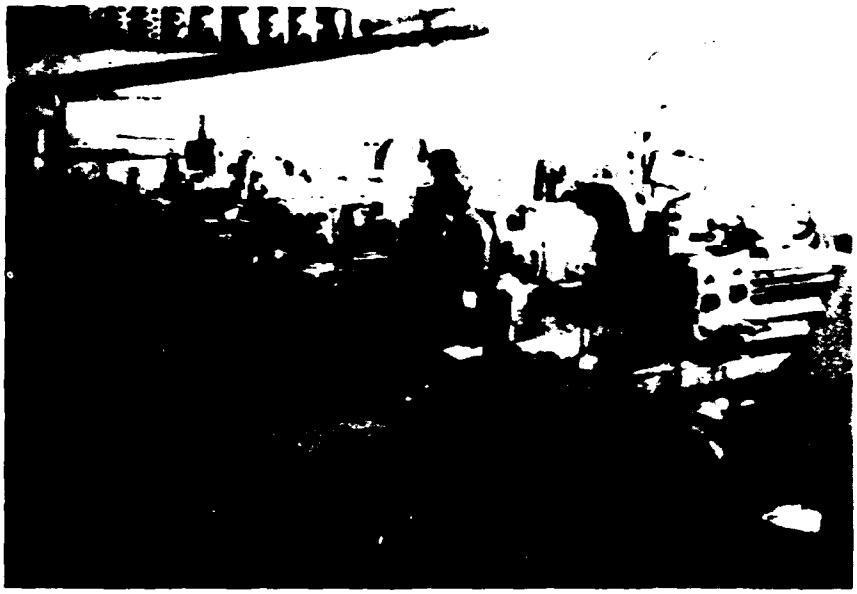
d) Distribution board need to be shifted to a common feed area for different equipment.

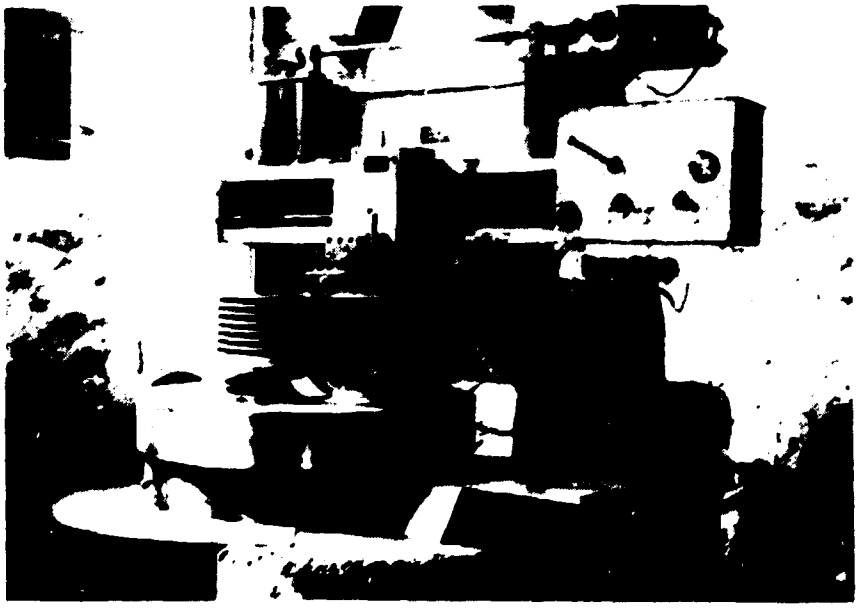
8.2. Preventive maintenance.

8.2.1. It is claimed that a shedule is drawn for yearly preventive maintenance work. All inoculators and seed vessels are supposed to be checked once in 3 months. All the fermenters are to be taken for preventive maintenance once in a year.

8.2.2. Vessels and vessel jackets are supposed to be tested by the police for pressure test and certified.

8.2.3. According to international norms, all pressure vessels depending upon usage are tested under 1.5to 2 times the normal working pressure and certified for usage. It is not very clear how the testing is carried out here and certified.





Manufacturing workshop

9. Laboratories.

There are different kinds of laboratories with different designations. But to simplify the recognition of the laboratories, the following terminologies are used. Within the antibiotic complex building there are individual seed preparation laboratories for individual antibiotics. These are described in 2.1, 3.1, 4.1, and 5.1. Other than this there are two laboratories, one for microbiological sterility and assay test and another for chemical analysis of media components and antibiotic analysis. These two laboratories are independant of a Quality control laboratory in an adjacent building, where raw materials and finished goods are tested and certified. These three laboratories are described here.

9.1. Microbiology.

9.1.1. The head of Kanamycin seed and culture laboratory is incharge of the work team for the sterility testing and antibiotic assay laboratory. The samples are collected from inoculators seed tanks and fermenters and are checked for sterility in the following manner.

a) The samples are streaked on agar slants of the following composition.

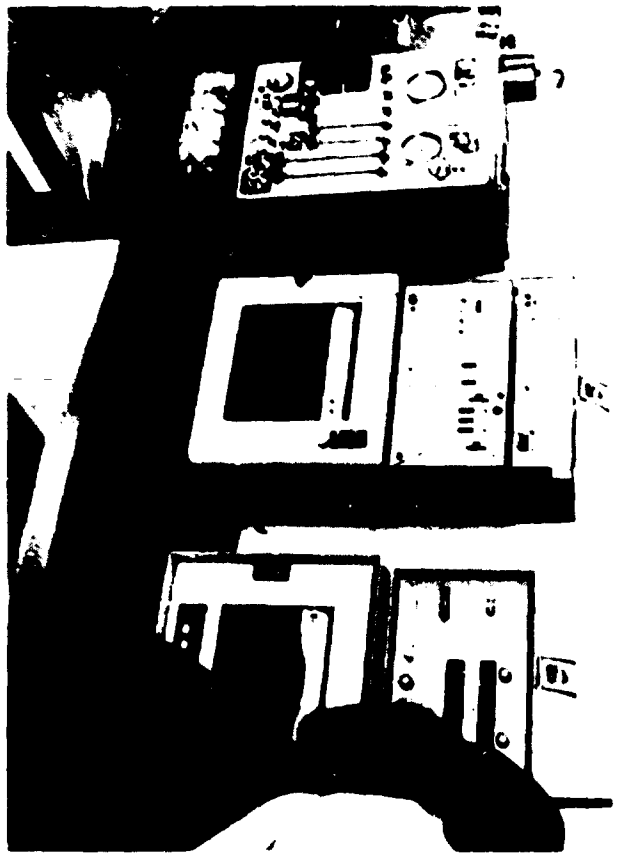
i) Beef extract .. 135 mg%
ii) Sodium chloride .. 0.3 %
iii) Potassium di hydrogen phosphate .. 0.1 ,,
iv) Glucose ..0.5 ,,
v) Agar .. 2.0 ,,
pH 7.2

The plates after streaking are incubated at 37°C and checked after 24 hours onwards for contamination.

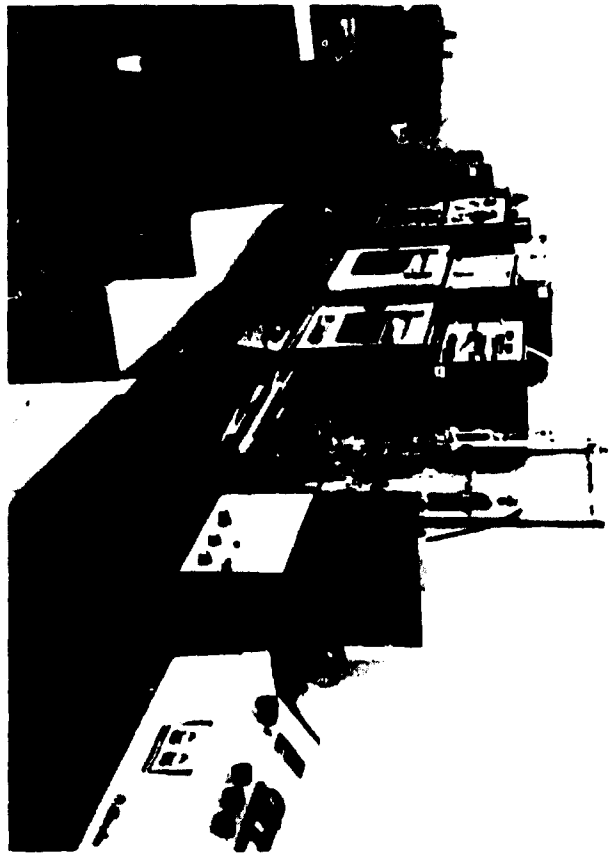
b) The same composition as in a) without the agar but with 0.004% phenolpthalein as indicator is taken in tubes as broth. Change of colour with pH indicate contamination of the samples.

9.1.2. Microbiological assay for antibiotics.

A wooden frame 25×25 cm with glass bottom and a depth of 1.5 cm serves as a bioassay plate. Basal agar medium 150 ml is placed at the bottom and the test organism in 75 ml agar is poured on top. Then wells of 8 mm diameter are punched on the agar. Samples of the different antibiotics are diluted proportionally to the concentration. Approximately 100 µl is put in to the well by a ss metal dropper attached to a glass tube provided with a rubber teat. After incubation at 37°C, the zones



O.C. Laboratories



Chemical testing lab

are measured next day for the assay.

a) Example Kanamycin assay.

Lower base agar has the following composition.

i) Beef extract .. 135 mg%

ii) Di sodium hydrogen phosphate .. 0.3 %

iii) Agar .. 2.0 ,,

pH 7.8

The upper part contains the same composition with the test organism Bacillus mycoides or B. subtilis. The test organism initially is streaked on agar in a Roux bottle. After proper growth, sterile water is added and the surface is scraped . This suspension is heated in a water bath at 70°C for 30 minutes. Already spores are formed. In order to get complete spore formation, the suspension is kept in water bath again for another 30 minutes. The contents are centrifuged and kept in cold as a stock.

20 ml of this suspension is taken in 100 ml water and the optical density is measured. Under specific dilution optical density is standardized and the suspension is taken and poured on agar to form the top layer.

b) In the case of Oxy tetracycline , B. subtilis L2 is the test organism. Except for minor difference in the composition of the uppper layer, the rest of the test is carried out in the same manner.

c) For Penicillin , B.mycoides HB or Staph. aureus 209 P is the test organism. The rest of the test is conducted in the same manner.

d) No test is carried out for Rifampicin assay.

9.1.3. Suggestions.

a) The broth sterility test should be conducted at broth levelin T.S. broth tubes. The Trypticase Soy broth after sterilization is a clear liquid and bacteria grow very fast in this liquid. One drop of contaminated actinomycetous broth if incubated at 37°C will show turbidity even in 6-8 hours time. Where as a sterile broth after incubation in T.S. broth will have only the small quantity of the mycelial suspension but no turbidity.

b) The agar test for sterility may be conducted on Yeast extract, Malt extract, Glucose medium, composition of which has been given to the personnel.

c) Assay methodology is crude due to lack of Polystyrene plates and standard pippetes.

9.2. Chemistry, (chemical analysis)

9.2.1. Equipment found in the laboratory are minimal for any kind of analysis. There are 3 calorimeters for analysis of antibiotics, 1 polarimeter, 4 pH meters

9.2.2. Sugar analysis is done by a very old method of iodine thiosulphate titration. Samples are hydrolysed by 6N HCL for sugar conversion.

9.2.3. Phosphorous and Ammonia nitrogen analysis are also primitive.

9.2.4. Complete new methodology for the analysis has to be incorporated. Either an autoanalyser or spectrophotometer will do for the analysis

a) For antibiotic analysis the need is for H.P.L.C. Analysis are time consuming and 20 samples of sugar are analysed in 90 minutes time.

9.3. Quality control.

9.3.1. This is the central testing laboratory for raw materials, and finished products. Number of instruments are limited and outdated. Many of them are more than 15 years old. Available instruments are:

a) Polarimeter
b) Calorimeter
c) pH meters .. 3 Nos
d) Conductivity meter
e) Sugar analyser
f) Moisture analyser
g) Solvent analyser
h) Carl Fisher apparatus
i) Spectrophotometer

Other instruments for testing in injectibles.

9.3.2. Quite a few of the organic reagents are imported. Only few of the inorganic reagents are available in the country.

9.3.3. Requires complete modernization, with new instruments. Refer 13.3

10. Warehouse and Stores.

10.1. Raw materials.

10.1.1. Many of thecommonly available raw materials are scarce and their supply need to be increased appreciably. Corn steep liquor though available , it is not in large quantities. Cane sugar which is very widely used by all countries, has to be imported from Cuba at the rate of $ 250/ ton. Even vegetable oils are scarce and their supply needs to be increased . Soyabean oil availability is low and it is imported(in addition to the local supply.), at the cost of $ 450/ ton. The prolonged winter months are a deterrant to the cultivation of any kind of crop. Hence vegetable oil supplies are limited. Corn flour, corn starch, soya bean flour are available as local supply. Glucose is available only to a limited extent and has to be imported. Most of the items are procured through trading companies from Hongkong or Singapore. Most of the salts like Magnesium sulphate, Sodium chloride, Ammonium nitrate, Ammonium sulphate, and others are procured locally.

10.1.2.Best production of Penicillin is possible with initial medium containing Lactose, Corn steep liquor and Hydrol. Shots are then added either with Hydrol(containing 50% sugar) and Phenyl Acetic Acid or with cane sugar and PAA. The choice of Hydrol and Cane sugar mainly depending upon the culture. Addition of Ammonium nitrate can be carried out depending upon analysis. As all these are not readily available, it is a big handicap.

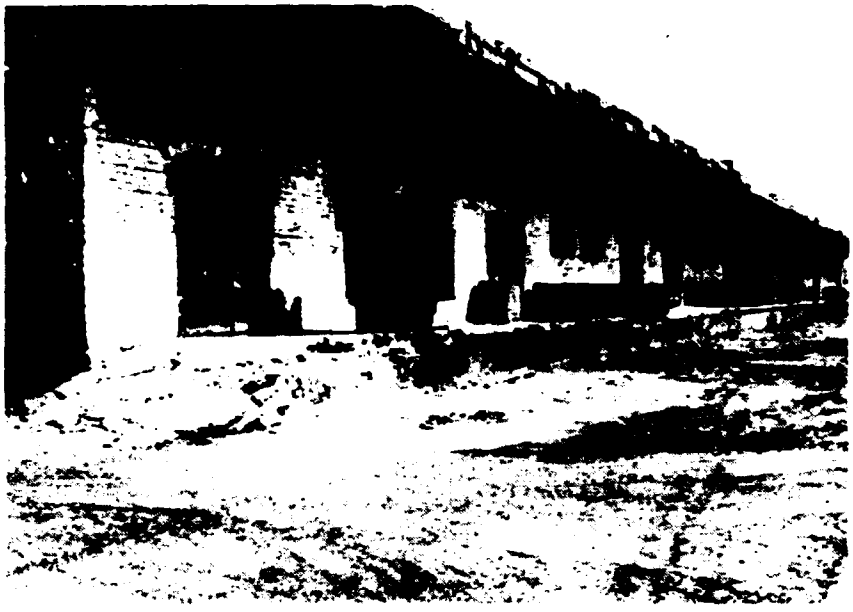
10.1.3. Oxy tetracycline production can be enhanced with the introduction of Pharmamedia and corn steep liquor in the medium. C.S.L. though may be available to some extent from local supply, there are no road tankers nor railway tankers available to transport the material.

10.1.4. Warehouse in in the backyard, with railway siding and unpaved roads. The divisions are as under:

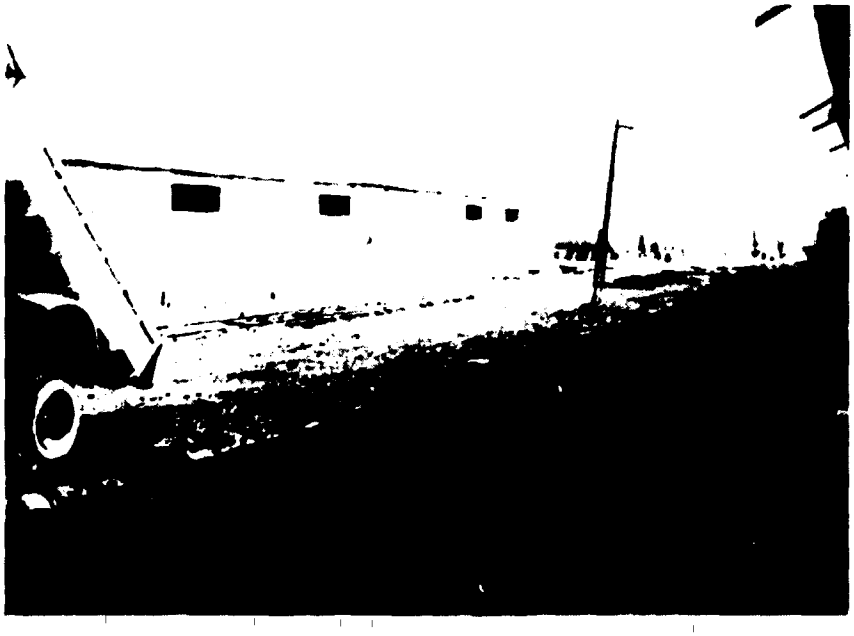
a) Finished products .. 1736 M^2
b) Raw materials .. 600 M^2
c) Equipment and spares .. 500 M^2
d) Salts and others .. 600 M^2
e) Smaller ones .. 400 M^2
Total .. 3836 M^2

The warehouse has sloping tin sheet roof and have mostly double doors for access.

10.1.5. Tanks of solvents, caustic soda, oil, methanol, liquid ammonia



Ware house



are all kept side by side on concrete base. There are more than 12 tanks.

10.1.6. Imported raw materials like soyabean oil, propyleneglycol, chloroform, isopropyl alcohol, phosphoric acid are kept in drums in closed sheds. No special tank farm is available.

10.1.7. Suggestions.

a) The solvent storage should be completely enclosed and isolated away from acid, alkali, and other storage tanks.

b) Drums containing oil and other solvents should be in an accessible area and away from each other.

c) Stacking of material should be very orderly and easily accessible.

d) No fire fighting equipment of any kind are seen near the area

10.2. Engineering spares.

10.2.1. Many of the flanges, complete air filters, pulleys, covers are manufactured in the manufacturing workshop for distribution to the individual sections.

10.2.2. Other parts like bearings, instrument spares, valves and other accessories are stored in the warehouse area.

10.3. Others.

All other material required for the workshop fabrications civil requirements, and the like are kept in the yard area. Diesel oil and prtrol tanks are also available for the factory.

10.3.1. The following trucks are at the disposal of the factory for the transportation of materials.

a) 10 ton .. 5 no
b) 8 ton .. 1 no
c) 4 ton .. 3 no
d) 2.5ton .. 8 no
e) Freezer car .. 4 ton .. 1 nO
f) ,, ,, .. 0.5ton .. 1 no
g) Tractors .. 3 no
h) Bus .. 1 no
i) Cars .. 2 no

11. Safety and controls.

11.1. Safety.

11.1.1. There is a central fire fighting brigade for the whole area, which can be called on phone as and when required. Within the factory there are fire tenders. This is a trolley with pump and hose connection. No other fire extinguishers were seen in the fermentation area. Though fire extinguishers of some kind were reported to be available in the down stream processing area by the staff.

11.1.2. Five persons who are trained in fire fighting are working in the plant. No other training is imparted to others.

11.1.3. Penicillin and Kanamycin mycelia are disposed off as feed additive. Oxy tetracycline mycelium is also dried and sent to cattle farm. Presence of these antibiotics in meat is a health hazard, and the use of mycelia as cattle feed should be avoided.

11.1.4. Comments and suggestions.

a) No safety glasses are worn by anyone in the whole plant. Even personnel working in the chemical labs are without safety glasses. Government should introduce safety regulations making it mandatory that all staff in the plant to wear safety glasses , and those in the work shop to wear crash helmets.

b) Metal staircases in mezzanine floors, outside tanks and other staircases need to be replaced.

c) Corridor lighting and lagging of pipe lines need attention for safety reasons.

d) Fire extinguishers of CO_2 , chemical and foam type for different kinds of fires are necessary.

e) General safety awareness seem to be lacking. Personnel working in the plant are to be trained in the emergency fire fighting proceedures.

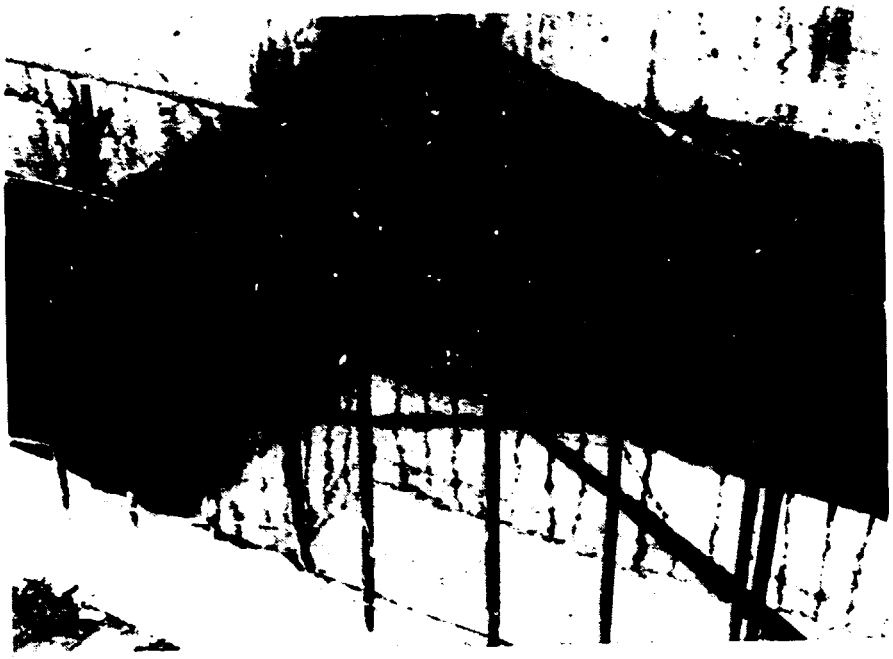
11.2. GMP.

11.2.1. GMP is very low. Quite a lot of general cleaning is required in many areas. Every thing is attributed to lack of funds. Many places and equipment like the broth overflow tanks, harvest tanks all need drastic modification to improve GMP standards. It is important for factory staff to bear in mind that so far as the manufacture of Pharmaceuticals are concerned cleanliness and hygeinic conditions are critical.

11.3. Effluent treatment.

11.3.1. Effluent discharge is roughly 250-350M^3/ day. There is a primitive plant for the effluent treatment beside the river. The initial c.o.d. is 800-1000 ppm. The effluent is taken in an open tank, 1-2% calcium hydroxide is added to bring up the pH to 9.0.Then ferrous sulphate 0.1-0.2 % is added. It is then sent to 2 precipitation tanks of 50 M^3 each. . From here the effluent is taken to an annular 600 M^3 double tank. Air is sparged through draft tubes for 48 hours and COD is brought to 150 ppm and then it is discharged in to the river. As the ambient temparature is low during 5 months, bacterial action is very low and the BOD is checked only occassionally.

11.3.2. A major modification of the entire system is warranted for efficient and effective treatment. Biological filters with controlled temparature, activated sludge treatment are to be carried to make the system effective.

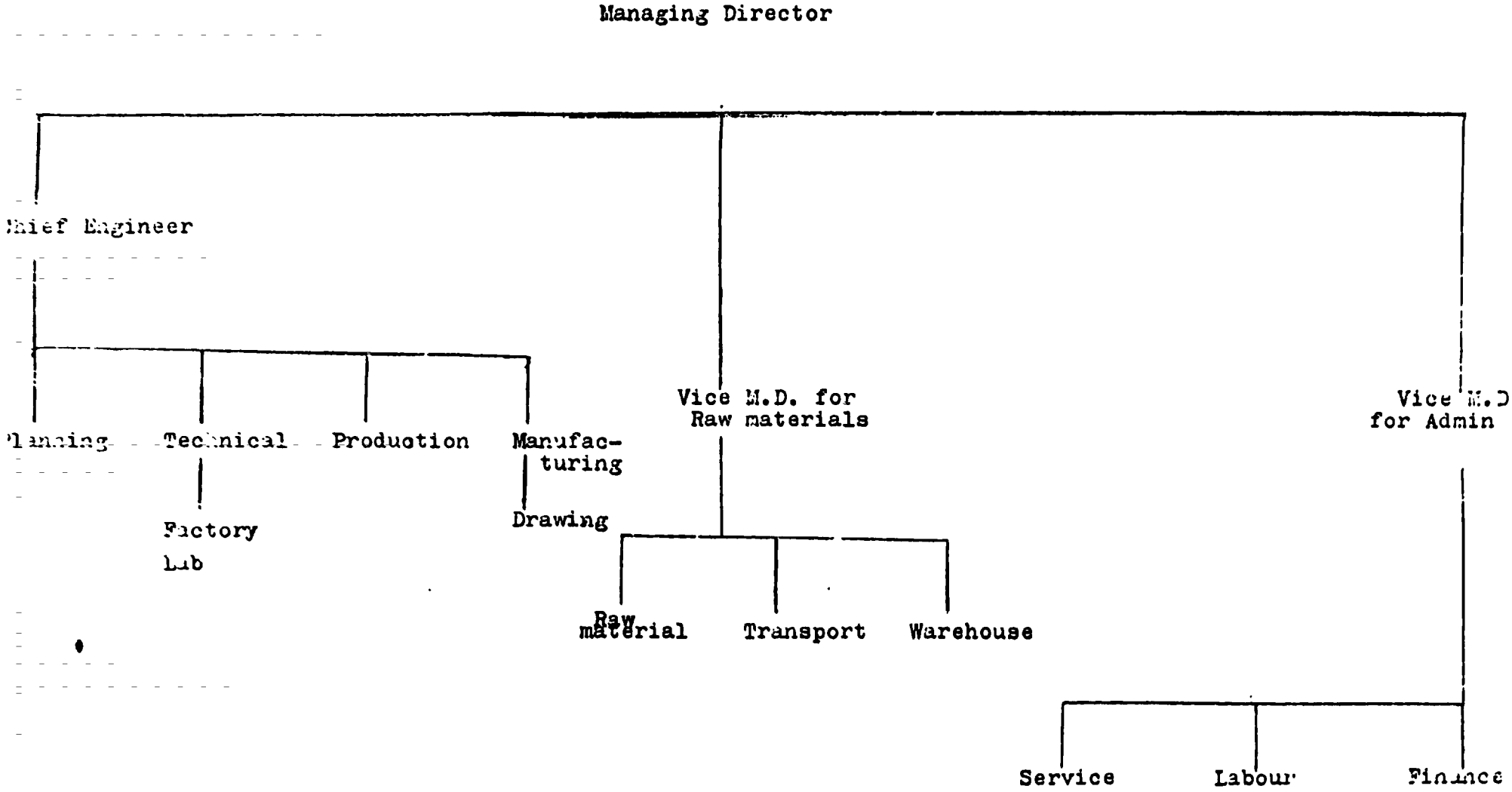


Effluent treatment

12. Personnel.

12.1. Present strength.

12.1.1. Organization chart.



12.1.2. Antibiotic plant. Employee breakup.

Product	Strain room	Seed	Fermen-tation	Purifica tion	Solvent	Activity	Special group	Total
Penicillin	2	7	11	27	10	3	13	73
Kanamycin	2	7	7	32	4	3	12	67
Oxy tetra-Cycline	2	7	10	22	3	3	10	57
Rifampicin	3	5	8	31	7	3	12	69
Total	9	26	36	112	24	12	47	266

12.1.3. Technical staff in antibiotic plant.

Designation	Penicill-in	Kanamycin	Oxy tetra cycline	Rifampi cin	Total
Engineers	6	7	3	5	21
Asst. Engineers	14	9	11	9	43
Total	20	16	14	14	64

12.1.4, There are 238 personnel in the accessory team. These include personnel of the following department.

Department	No of persons
a) Derivative	20
b) Hygeine & nutrition	18
c) Sewage disposal	15
d) Packing	95
e) Repair	17
f) Power lines	5
g) Inspection	6
h) Office managers	10
i) Testing laboratories	33
j) Solvent and other Pen. derivatives	11
k) Machine operators	8
	238

12.1.5. In the industrial laboratory consisting of R&D, Pilot plant and accessory sections there are 92 persons working in different areas. Sections here include microbiology, purification, pilot plant, compounding and inspection.

12.1.6. Engineers and Asst. Engineers.

a) This group includes persons who have graduated with a degree in specialised subject. This is a general term and in this context includes persons working in laboratories, fermentation, recovery, engineering maintenance and services. After their initial graduation they are further trained in the capital city for their specialised jobs.

b) Those who have finished school and joined in the jobs attend part time college/ night courses and improve their qualifications.

c) There are different grades in the profession of engineers.

i) Those who have graduated and joined are in grade 6.

ii) After 3 years, they have to pass examination conducted to go up to grade 5.

iii) Every 3 years subsequently exams are conducted to check efficiency and the personnel are up graded to grade 4, 3 and so on, to 1

12.2. Suggestions.

12.2.1. Many of the staff even at the top level are not exposed to the latest technological advancement in this field. It is worth while that a technical team comprising of the Managing Director, Chief Engineer and members of the Bureau in charge of the complex as advisers should be taken to advanced plants. Plants located in West Germeny, Holland, Denmark, and Spain are quite advanced. The proposal is that UNIDO should get permission from these different countries, so that the team can visit as many plants as possible in advanced nations. In the case of developing countries they can visit India ,Yugoslavia, Indonesia and the like. This visit may enable them also to implement certain of the suggestions given in the report, as they are required for basic modification.

12.2.2. Training for technical staff.

Some of the technical staff in grade I and II are very well versed in their sphere of activity, and need encouragement and exposure. It is proposed that atleast 3 to 4 persons from each section comprising of fermentation, purification, laboratories and 1 each from maintenance and services are to be taken up for training in the initial stages. Training of this staff in the advanced countries may pose a problem. In that case even exposure to different plants in developing countries and training in them will help to a large extent. Subsequently feasibility of training for further batches should be looked into on the long term perspective. The gap in knowledge is very large and it is hoped that atleast something can be given to them in stages.

13. Overview.

13.1. New products.

13.1.1. With the present set of fermenters and accessories productivity of new products if introduced, may pose a problem. In order to introduce new products in the setup, quite an amount of modification are necessary. After installation of new equipment and improving the lot of vessels and accessories, it is better new products are introduced.

13.1.2. The logical step next to the Penicillin production is to go for Ampicillin production. Conversion of Pen G to 6 APA by enzymatic conversion, followed by semisynthetic pathway willbe the most ideal step. This process can be introduced. This requires a new setup.(13.4.)

13.1.3. Erythromycin is already being worked out by the R&D team. Though the yields are low, if new medium, technology and equipment are introduced this may turn a new leaf. In the international market the maximum yield is 6-7 gm/lt. This has a wide market in Asia and will have a very good market potential.

13.1.4. As the plant is already producing Oxy tetracycline active against gram+ and gram- bacteria, introduction of derivatives may be easy. More active compounds like demethyl tetracycline were produced by chemical modification of tetracycline. Likewise introduction of Methacycline or Doxycycline from CTC may be a better derivative than OTC iteslf and market potential is high.

13.1.5. It is worth while considering the introduction of Cephalosphorin C in the long run. It is not a complicated fermentation as Rifampicin and recovery after purification are very good. The technology is available in Europe and introduction after installing new equipment and upgradation can be considered.

13.2.Technology upgradation.

13.2.1. New strains with complete technological paremeters in the different areas as the following are available.

a) Strain maintenance
b) Medium for culture slants
c) Fermentation and seed medium in different stages
d) Additions during the cycle
e) Parameters during the cycle
f) Product recovery

13.2.2. The latest productivity values in the international area for two of the antibiotics of the Gunchon plant are as under:

a) Penicillin .. 55000 u/ml to 65000 u/ml .. 33 to 39 gm/lt

b) Oxy tetracycline .. 15-18 gm/lt

Rifampicin and Kanamycin are to be checked for the productivity at international level.

13.2.3. The following companies are known to sell the technology as explained in 13.2.1.

a) J.C.P.Martin & Associates. Channel Islands .GR. BR

b) Pan Labs Inc. Taiwan

13.2.4. These technologies are quite expensive in the market. Prices seem to be negotiable and payable in instalments. The first instalment on receipt of the culture strain and the technology , the second on achieving 75-80 % of the rated productivity and the last on achieving full productivity.

Cost will work out to approximately the following:

a) Penicillin .. 55000 u/ml .. $ 300,000 TO 500,000

b) Erythromycin .. 6 gm/lt .. $ 300,000

c) Tetracycline .. 25 gm/lt .. $ 300,000 TO 400,000

d) Ceph. C .. 25 gm/lt .. $ 500,000

13.2.5. For the scaleup work from shaken flasks to fermenter, it is essential to have the best Pilot plant facilities. This is very critical stage operation and it is difficult to establish the correct parameters in large fermenters unless it is studied in the pilot plant in detail. Hence it is very essential to import a set of Pilot plant fermenters complete with all accessories and controls for the detailed study of culture parameters in fermenters. (refer 13.3.6.)

13.3. Equipment.

13.3.1. General.

The present condition of the plant is like other plants in Europe or advanced countries in late fifties or early sixties. Due to paucity of funds or other reasons the plant is suffering enoumously. The fermenters and many other accessories might have been scraped if the plant is located in industrialised countries. Some of the vessels may find some use as mixing tanks, but many others are not worth writing about. It is essential that in order to make the plant working upto certain level of standards, funds have to be allocated to procure equipment needed badly. If at a later stage the upgradation of the plant is taken in larger perspective, the plant can survive. With the present technology, equipment, and infra structure it is bound to receede in the long run due to plant overall degradation, rather than improve.

The following equipment are needed and are suggested for the upgradation of the plant to a major extent. The chapters are diveded in to several sections starting from the laboratories to the production fermenters in the order of workup.

13.3.2. Laboratories. Microbiology

Equipment	Manufacturer/ make	Country of origin
a) Microscope	Olympus/ Nikon	Japan
b) Laminar flow bench	Klenzaids or Fisher Scientific	India U.S.A.
c) Shakers i)single speed ii) incubator iii) triple decker	Infors or B.Braun	Swizerland West Germany
d) Pippetes i) normal ii) adjustible iii) dilutor	Eppendorf	West Germany
e) Polystyrene plates and petri dishes	...	West Germany or Holland
f) Centrifuge	Sorval or I.E.C.	U.S.A.
g) B.O.D. Incubator	Fisher Scientific Arthur Thomas	U.S.A.
h) Lyophilizer	Virtis Co.	U.S.A.

13.3.3. Chemical laboratories

a) Spectrophotometer	Fisher Scientific	U.S.A.
b) Auto analyser	Hewlet Packard	U.S.A.
c) H.P.L.C. complete system with auto sampler	Shimadzu or L.K.B.	Japan Sweeden
d) Rotavapors 1-3 lt 20 lt	Buchi	Switzerland

13.3.4. Fermentation control

a) Temparature controller & recorder	Marubichi or B.Braun	Japan West Germany
b) pH controller & recorder	,,	,,
c) DO_2 controller & recorder	,,	,,
d) Rotamerers for inoculators & seed tanks	,,	,,

Equipment	Manufacturer/ make	Country of origin
e) Anti foam control	Marubishi or B.Braun	Japan West Germany
f) Volume indicator	,,	,,

13.3.5. Harvest

a) Rotary vacuum filter or centrifuge	Westfalia or Dorr Oliver	West Germany India
b) Pre coat filter	Dorr Oliver	India
c) Counter current extractor	Potbielnik or Westfalia	U.S.A. West Germany
d) Rotavapor : 50 lt	Buchi	Switzerland

13.3.6. Pilot plant

a) Pilot fermenters 2 of 50 lts	Marubishi or Chemap	Japan Switzerland

13.4. Expansion.

13.4.1. Already plans are afoot for the expansion of Kanamycin production. The following vessels are being erected.

a) Inoculators .. $1M^3$ .. 4 Nos . stainless steel
b) Seed tanks .. $6M^3$ .. 4 Nos . ,, ,,
c) Fermenters ..$35M^3$ .. 4 Nos . carbon steel.

All of them will have the same kind of design and control as the existing fermenters.

13.4.2. There are plans for setting up Ampicillin production, details of which are not available as yet.

13.4.3. It is better to infuse funds at this stage during the expansion of Kanamycin for the purchase of fermentationcontroller equipment like temparature, pH, DO_2, antifoam and accessories. Then the fermenters will run with enough controlled paremeters for better productivity.

13.4.4. For setting up expansion of any kind of activity there is no darth of space. Some of the vessels and accessories can even be modified to accommodate new products.

Kanamycin expansion

PART 14. CONCLUSIONS AND RECOMMENDATIONS

General condition of the plant is poor. The building and equipment are very old and need upgrading.
The following are the yields of antibiotics fermented at present:

Penicillin 17000 u/ml

- Harvest volume m3 fermenter - 10.5 m3
- No. of batches run per year/fermenter - 36.5
- No. of batches run per year/6 fermenters - .219
- yield/fermenter - 178.5 mlds
- total yield in 15 m3 fermenter - 39091.5 mlds
- volume at harvest in 20 m3 fermenter - 14.5 m3
- yield/fermenter - 246.5 mlds
- 36.5 x 2 fermenters - 73 batches
- total yield in 15+20 m3 fermenters - 57086 mlds
- after purification at 61.5 % - 35107 mlds

Kanamycin 4 gm/lt

- volume at harvest in 15 m3 fermenter - 11 m3
- yield/fermenter - 44 kg
- No. of batches taken per year/fermenter - 72
- " " " " x 2/fermenter - 144
- total yield in 15 m3 fermenters - 6336 kg
- volume at harvest in 20 m3 fermenter - 14
- yield/fermenter - 56 kg
- 72x2 - 144 batches in 20 m3 fermenter - 8064 kg
- volume at harvest in 35 m3 fermenter - 22 m3
- yield/fermenter - 88 kg
- No. of batches in one fermenter - 72
- yield in 35 m3 fermenter - 6336 kg
- total yield in 15+20+35 m3 - 20736 kg
- total yield after purification at 58 % - 12.026 m.t.

Oxytetracycline 10 gm/lt

- 10.5 m3 in 15 m3 fermenter yield - 105 kg
- 39 batches x 6 fermenters - 234 batches
- total yield in 15 m3 fermenter - 24570 kg
- 22 m3 in 35 m3 fermenter yield - 220 kg
- 39 batches /year - 8580 kg
- total yield in 15 m3+35 m3 fermenters - 33150 kg
- total yield after purification at 61 % - 20.221 m.t.

Rifampicin 3.5 gm/lt

- 11 m3 in 20 m3 fermenter yield - 38.5 kg
- No. of batches taken per year - 21x4 fermenters - 84
- total yield in fermenters - 3234 kg
- total yield after purification at 25 % - 808.5 kg

RECOMMENDATIONS

Short term suggestions for improvement

1. Air system needs modification. The erstwhile practice adopted with cotton and activated carbon, should be dispensed with. Either emulsified staple fibre (esf) glasswool filters or absolute air filters have to be installed.

2. Air sparger in all cases are to be modified. Ring sparger with holes at the bottom and top have to be installed for better oxygen distribution.

3. Agitator blades have to be modified to rushton flatblade turbine. Basic design has been given already,

4. Agitator shaft support at the bottom of the fermenter need modification with teflon sleave.

5. The distance between the blades should be adjusted taking the volume into consideration.

6. Harvest line shoud be modified with the bottom valve, bottom drain valve with steam and condensate line connections in between.

7. Vent line has to be provided with srubbing device and also with steam connection.

8. Media mixing tank, which is at present only of 3 m3 capacity, should be enhanced to 12 m3 so that at least 1/3 of the volume of the big fermenter capacity can be fulfilled.

9. Media mixing should be improved upon. Soja bean flour and corn flour are to be sieved with 500 u sieve. Starch should be hydrolysed before sterilization.

10. Controllers for the following are to be procured and installed :
a) temperature controller +/- 0.2 C
b) pH indicator controller
c) DO2 indicator controller
d) antifoam probe with addition vessels and with solenoid valves/peristaltic pumps additions.

11.
a) harvesting volume to be improved up to 1:0.8 volume of the vessel
b) water addition after harvesting 2 m3 to 5 m3 of the broth during the final stages will improve productivity.

12. New media components have to be introduced:
a) lactose and hydrol for penicillin
b) pharmamedia and corn steep liquor for OTC and Kanamycin

13. Fermenter carbon steel vessel has to be improved upon by cladding with 2 mm sheet of SS316 inside the fermenters.
In the same way agitator blades and sparger are to be made of SS316 material.

14. Aeration rate has to be increased for penicillin fermentation up to 1:1.2 during the fermentation cycle.

15.
a) Broth analysis for sugar and PAA in penicillin fermentation should be done every 2 hours and additions monitored accordingly.

16.
a) culture maintenance should be improved upon
b) preservation of vegetative mycelium in liquid nitrogen should be introduced.

17. Lump sum addition of glucose or sucrose inthe initial medium in Refampicin should be avoided. To avoid catabolic repression only 1 to 2 % should be taken initially and the rest should be aded gradually after analysis for sugar.

18. Direct spores inoculation in the first stage should be avoided (kanamycin). This prolonges the lag phase growth in the inoculators (first stage). Pool seed from flasks should be utilized for seeding the first stage inoculators.

19. Initial use of glucose 0.5 to 1 % should be checked for OTC as there is no readily available sugar source for the culture.

20. Liquid ammonia 25 % should be filtered through zeit's filter before use in fermenters.

21. Addition of any NAOH through air line in Rifampicin should be discontinued. A separate SS316 line should be installed.

Immediate equipment required

1. air compressors 200 m3/min - 2 hrs

2. absolute cartridge filters for air

3. air receiver of 70 m3 capacity

4. efficient water renewal by basic supply to cooling coils in air system.

5. a) temperature indicator controllers
   b) PA " "
   c) DO2 " "
   d) PCO2 " "
   e) antifoam probe with complete control system

6. HPLC

7. rotary vacuum filter

8. counter current extractor

Training and technical visit

a) Technical team should be sent to developed countries and developing countries (chapter 12.2)

b) Engineers and test engineers should be trained in developing countries

Long term plan

1) Improving penicillin productivity in the plant by adquiring new technologies and assistance and to start the 6-APA and ampicillin production.

2) To improve the OTC production and start the Doxycyclin technology transfer.

3) Erythromycin at 6-7 gm/lt

4) To begin the studies for Cephalosporin C technology transfer and its semisynthetic derivatives.

Technology upgrading

Better strains with complete technology for fermentation and recovery are to be processed (see chapter 13.2)

Equipment

A detailed list of equipment needed in the long run has to be submitted (see chapter 13.3.).