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19824

35 p.
diagrams

FINAL REPORT

SECOND OPINION AND RECOMMENDATIONS ON VIE/86/016

BY ULF STRENGER;

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1992-08-24

Abstract

The technical documentation of the project VIE/86/016 has been reviewed. The findings and conclusions from the review and recommendations to the future execution of the project are presented in this report. Detailed recommendations on each piece of equipment as well as on the equipment lay-out are given.

A literature review has been performed and the conclusion is that the proposed methods for the production of pancreatin and chymotrypsin/trypsin are in principle in accordance with modern literature.

With some additions of new and some changes to the present equipment three new products are included.

This report replaces the interim report, 1992-07-21.

Contents

1 INTRODUCTION

1.1 Background

The project, VIE/86/016, is now in the stage where detailed engineering is being finished. However, before initiating the construction of the locally produced equipment, the necessary building and installation works, the Unido Country Director requested a qualified technical evaluation of the project (a second opinion), starting from the selected technologies and thus the full set of designs/drawings for equipment construction and installations.

1.2 Aim of the work

To review in full detail the existing technical documentation for the project. To check existing detailed construction (equipment and buildings), electrical, instrumentation and mechanical installation designs/drawings necessary for guiding the local equipment fabrication, installation/erection of the pilot plant and in particular, to examine basic process flowsheets, mass and energy balances and advice upon up-to-date technologies. To check wether the pilot plant can accommodate additionally such products as thyroid powder, magnesium biliar salts and peptone and if not, to determine what additional equipment and adaption would be required to do so.

1.3 Work plan

One week of literature search and study of part of the technical documentation of the project by the team leader and the process engineer in Sweden. One day of briefing in Vienna with the back stopping officer (Ms. O. Valdes) the team leader (prof. F. Setterwall) and the process engineer (U. Strenger). Mission in HoChiMinh city by the process engineer, backed up by the team leader through telephone and fax. One week in Sweden completing the work and writing this final report basing on the the interimreport presented already in Vietnam.

2 CONCLUSIONS

The proposed methods for the production of pancreatin (two methods) and of chymotrypsin and trypsin (one method) follows the general procedure for the production of industrial enzymes from animal organs. There are some differences though: (1) no activation step and no defatting step in the production of soluble pancreatin and of chymotrypsin and trypsin, (2) no milling of the product.

A literature search resulted in five alternative methods for the production of pancreatin and of trypsin. All methods are different, but show similarities. No mean of comparing the quality and yields of the different methods was available, why it was not possible to recommend one method before another.

Thyroid powder, magnesium biliar salt and insoluble pancreatin are included in the pilot plant. One new vessel, some ex-proof motors and other electrical installations, jackets, new connections, a system for regeneration of petroleum ether using the present distillation column and a cool water system of 0 °C are needed. Thyroid powder is the product that requires the most changes.

The flow sheets and the equipment, that is going to be manufactured by a local company, has to redesigned and redrawn according to the recommendations in appendix 2.

3 FINDINGS

3.1 Sources of information

The findings are based on the three PTE reports, equipment drawings, civil engineering drawings, data sheets from foreign manufacturers, the fellowship report, study visits and discussions with the local staff. Apart from this a literature search and study was made in Sweden.

The technical documentation of the project is scattered over these three reports, faxes and letters and therefore difficult to follow. It is also incomplete concerning the foreign equipment.

3.2 Production schemes and material balances

The findings from the study of the production schemes and material balances presented by the PTE in May 92 are found in appendix 1.

Recommendations on production schemes and material balances and equipment for the additional products are also given in appendix 1, where new production schemes and material balances are drawn and presented.

3.2.1 Capacity

Because of the too small size of the vessels in pancreatin and because of the shape (too high length to diameter ratio) in chymotrypsin/trypsin production, the capacity of the plant is approximately 70 % of the intended capacity. It is not possible to decrease the amount of extraction solvents if the same product quality is to be expected.

3.3 Utilities

3.3.1 Electricity

From a summary of all available data on power consuming equipment it is found that the required power is 320 kW if all equipment is used at the same time. The transformer's output is 630 kW. Data were missing on the following pieces of equipment: water cooling unit, cool room, air drier, ventilation system, distilled water apparatus, demineralized water apparatus and all stirrers that are not yet sufficiently defined.

3.3.2 Steam

Steam is used in the distillation and in the sterilizer 01.9. The steam consumption is 146 kg/h and the production capacity is 380 kg/h.

3.3.3 Light

It is checked that the fluorescence lamps in the rooms 8, 9, 12 are explosion proof on the drawings. If thyroid powder is going to be produced, there have to be ex-proof installations in rooms 11 and 16.

3.3.4 Water

It is not known whether the tap water is clean enough to be used in the production. In the water quality certificate on PTE's report in November '91, p. 14, the contents of germs, BOD, COD etc. are not mentioned. There are 56 mg solids/l. The certificate is not signed.

It is not known whether the warm water is suitable to clean the equipment. The required quantity of warm water is not checked.

The required amount of cool water and demineralized water has also to be checked.

3.4 Equipment

3.4.1 Locally purchased equipment

From the findings from the study of the drawings, recommendations are given on each piece of locally purchased equipment in appendix 2.

3.4.1 Foreign equipment

Missing documents

Documents describing the air drier, the ventilation system, the distilled water apparatus and the demineralized water apparatus were missing at the time of the mission.

Kavalier distillation column

(a) Since glass parts are fragile, spare parts have to be purchased as soon as possible. Nothing is mentioned about spare parts in the quotation.

(b) The column is not capable to give 96% w ethanol because of the azeotrope, which has to be broken by means of lower pressure (not possible in this column), extractive or by azeotropic distillation (requires more equipment).

Meat cutter RM83
There seems to be no problems with this piece.

UNIS process control
For the control of the distillation column, the pressure has to be known if TI is intended to give the concentration. It's not clear if this is the case. Reflux ratio should also be possible to control. Since detailed specifications of the equipment should be in the cases (boxes) that were in the harbour by the time of the mission, this couldn't be checked. This should be carefully checked by DICI considering the comments given in this report.

Other findings and recommendations concerning the control system are presented in appendices 1 and 2.

Rina centrifuge
The label is 03.4. The capacity is only 25 l., which has to be taken into consideration while writing the operators manual for the plant.

Lampart pressure filter
This piece has only two sockets, 100 mm. This is not consistent with PTE's report November '91 p. 40. Recommendations are given in appendix 2.

Water boiler
There seems to be no problems with this piece.

3.5 Equipment lay-out

The findings form the study of PTE's report November '91 p. 44-46 resulted in the recommendations which are given in appendix 2.

3.6 Civil engineering

3.6.1 Electricity lay out

The drawings of the electricity lay out from the civil engineering organization have not been checked in detail. All motors and pumps are explosion-proof, where it was required when no additional products where considered. For the production of thyroid

powder though, the electrical installations in the rooms 11 and 16 have to be ex-proof. No electricity has yet been installed in the premises.

3.6.2 Reinforcements

The reinforcements of the ceilings are already built.

3.6.3 Ventilation and air conditioning

The air conditioning units (10 units) are placed in the rooms where they are needed. There are no units for the rooms where acetone is being handled. It still has to be checked whether there has to be ex-proof ventilation in the rooms where petroleum ether (for the thyroid powder) is being handled. The drawings on the ventilation system, Janka Radotin, were incomplete and not checked in detail. This should be checked by national authorities and DICI and if necessary, completion should be requested from the supplier.

3.6.4 Drainage

Drainage is not planned to be installed in all rooms where production is taking place, but it has to be as well as in the lab. This is necessary for cleaning with water.

3.6.5 Working conditions

In the lab and in the production rooms emergency showers and eye cleaning showers should be installed.

4 REFERENCES

(1) Fryda J., February '91, technical report
(2) Fryda J., June '91 technical report
(3) Fryda J., November '91 technical report
(4) Scedrov O., December '91 CTA report
(5) Labortechnik, invoice, 911118
(6) Food machine engineering works, Bratislavia, catalogue, RM 82
(7) Rina, catalogue
(8) AVP Anhydro A/S, Instruction manual
(9) Bason drawings: 01.2, G1.3&03.2, 01.4, 01.6, 2*01.7, 2*01.9, 01.10, 01.11, 02.2, 02.3, 02.4, 03.1, 03.3, 03.5&7, 03.6, 03.8, 04.1, 04.3, 06.4, 06.6, 09.3, 09.4
(10) Civil engineering organization's drawings: group 1,2 and 3
(11) Fellowship report (hand written)
(12) Miscallenous letters and faxes
(13) Kavalier quotation
(14) UNIS technical report, 1991
(15) Lampart invoice, 1991-02-14
(16) Janka Radotin one drawing.

References to the literature study are given in appendix 3.

PRODUCTION SCHEMES AND MATERIAL BALANCES

All processes in this appendix are also described in the technical documentation available listed in the reference list.

Contents

1 Soluble pancreatin

The material balance is reduced by 30 % compared to the material balance in PTE's block scheme of May-92 and is shown in the block scheme. The acetone balance in the May-92 block scheme is not correct since the acetone losses must be smaller if the distillation column is approximately correctly specified. In the block scheme presented here and in the PTE's block scheme the assumed concentration of acetone in both cases is 98 %w. The solids contents in the local pig pancreas should be determined by the trainees /technical project personnel. In general, the total solids content ranges between 14 - 20 % w. The PTE's block scheme is in accordance with this.

The steam and cooling water should also be reduced by 30 %. The electricity is used to power the stirrers and should remain the same. Steam and cooling water are also produced from electricity , but the power required by the boiler and by the chiller is not yet specified.

2 Insoluble pancreatin

For the production of insoluble pancreatin there are the following requirements on the equipment:

(1) One new vessel, labeled 01.12 in the block diagram:

(a) jacket connected to cw and cwc

(b) TIC for keeping +12°C. The literature study showed that 4°C is used sometimes. If this would be possible in this method, it would

be easy to put this vessel in the cold room and there would not be any need for jacket etc. This has to be investigated further.
(c) light and sight glass for the decantation
(d) hole on top for easy feeding, decantation and emptying of the remaining pancreas.
(e) 50 l. and anchor stirrer.

(2) 01.4 (a) jacket connected to cw and cwc
(b) TIC for keeping max +5 °C
(c) hole on top for feeding

(4) 01.11 (a) has to be designed so that it is possible to cool the acetone to + 5°C.

(5) 02.6 New decanter in the regeneration system for the removal of fat from the waste water. The same decanter should be used in the thyroid process.The design should be made by DICI.

The process is described as follows:
(1) Grinding of the pancreas.
(2) Activation with NaCl(aq), CaCl2(aq) and pancreatin as written in the block diagram. The temperature should be +12 °C all the time. The process starts with 15 minutes of stirring. After that, standing for 24 hours.
(3) Extraction of fat from the pancreas in the jacketed vessel 01.4 with acetone (5°C) cooled in 01.11. Pancreatin remains in the meat.
(4) Filtration in the pressure filter 1.5. The filtrate is acetone with fat and is circulated to the regeneration unit which is completed with the decanter 02.6. The fat can if there are facilities be used in the production of soap. If there are no such facilities, we suggest destruction of the fat by means of burning. The solids are recycled to 01.4 and the extraction is performed again etc.
(5) After the third extraction/filtration, the solids (product) are dried in the drier 01.7.

3 Trypsin and chymotrypsin

The material balance should be reduced by 30 % compared to PTE's block schemes of May-92. This is done in the present block scheme.

The "Cuban" method, described in the fellow's report, would require the following changes in the equipment:
(1) It has to be possible to maintain 0-5 °C in 03.1. It is possible with the changes.
(2) There has to be one refrigerated centrifuge (2700 RPM). Since the centrifuge 03.4 is jacketed, this could be refrigerated to max 5°C, but the RPM is lower.
(3) A jacket on 03.3 and on 03.6 to make it possible to maintain 0-5 °C. This is not recommended elsewhere.

The "Cuban" process is described as follows:
(1) Grinding of 35 kg frozen pancreas which have been treated with H_2SO_4(aq)
(2) Extraction with 0.25 N H2SO4 (70 l.) at max 5 °C for 10 hours.
(3) Centrifugation with a refrigerated centrifuge .
(4) The liquid is transferred to 03.3 and the solid is recycled to 3.1 where it is extracted again.
(5) The mixture is centrifugated again. The solid is waste pancreas and is sterilized.
(6) The liquid is transferred to 03.3 where salting out of impurities is carried out with 26.25 kg $(NH_4)_2SO_4$. Stirring 1 hour and standing 12 hours at 5°C.
(7) Centrifugation in refrigerated centrifuge.The solids (waste) are taken away and to the solution 20.3 kg $(NH_4)_2SO_4$ is added in 03.6. The crude proenzyme is then salted out during 1 hour
(8) Centifugation in a refrigerated centrifuge.The liquid is transferred to sewage and the solid to laboratory treatment.
The block scheme describes the process described by the PTE.

4 Magnesium salt of bile

For the production of magnesium salt of bile there are the following requirements on the equipment:

(1) 03.1	(a) jacket connected to steam and steam condensate as well as to cw and cwc.
	(b) TIC to steam - might be manual.
	(c) Connection to N_2 in order to pressurize the vessel.
(2) 03.2	(a) Connection to sewage.
(3) 01.7	(a) Connection to sewage

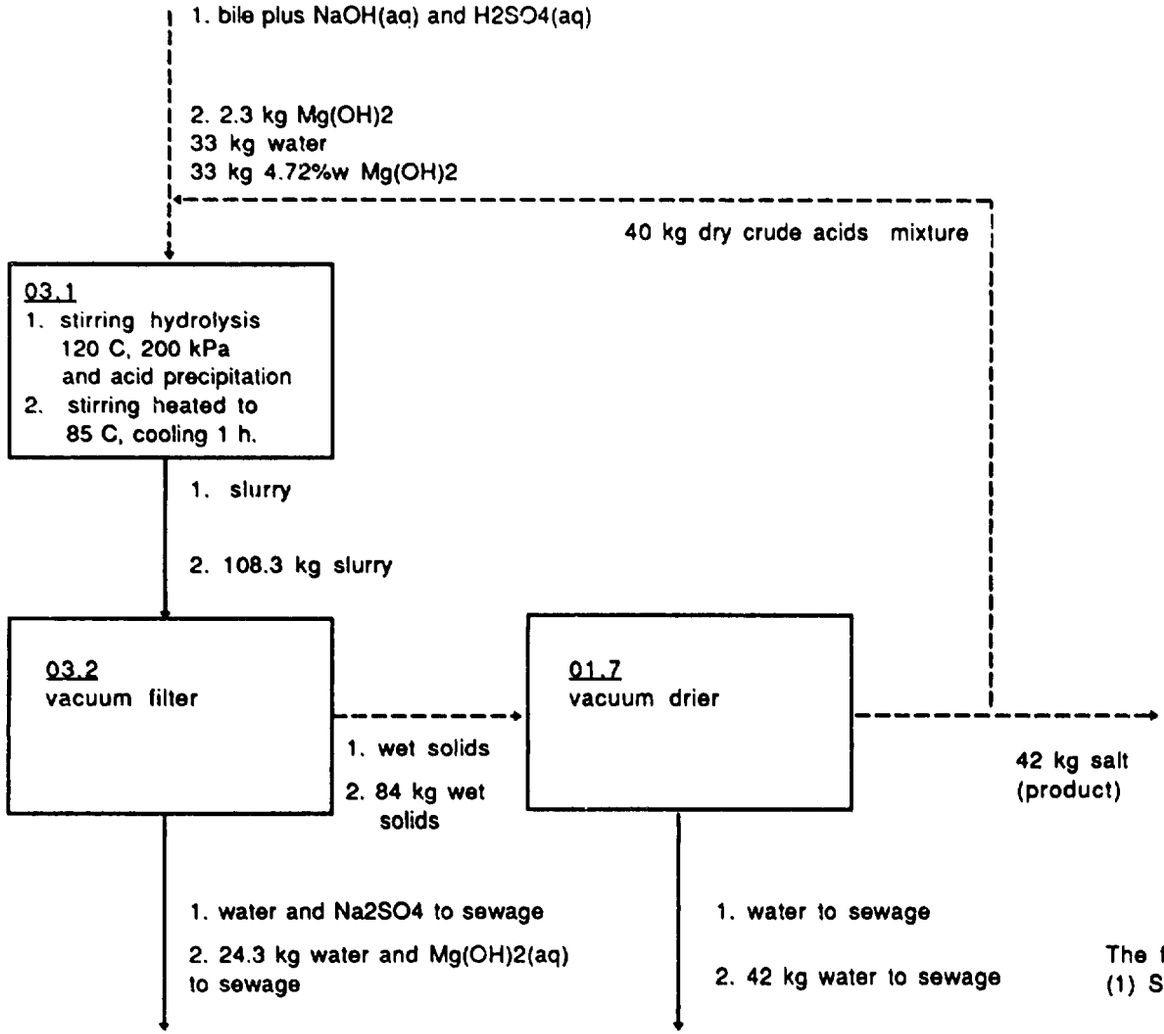
The process is described as follows:
(1) Bile is hydrolysed with NaOH in vessel 03.1. The reaction takes place at 120 C under pressure which has to be given by PTE or CTA. (2) After basic (NaOH) hydrolysis and acid (H_2SO_4) precipitation of the bile acids, the crude mixture is filtered on the vacuum nutch filter 03.2. (3) The solid is dried in the vacuum drier 01.7. (4) The dried choleic and desoxycholeic acids crude mixture is transferred back to vessel 03.1 where it is treated with 2.3 kg of Mg(OH)2, 33 kg water and 33 kg of a 4.72 % suspension of Mg(OH)2 in water. The mixture is heated up to 85 °C with stirring and is then left for cooling approximately one hour. (5) The slurry is transferred by means of gravitation to the vacuum filter 03.2. The liquid goes to sewage and the solids is transferred to the drier 01.7. (6) After drying in 01.7 the product is 42 kg of magnesium salt or bile.

In order to make the material balance complete the required amount of NaOH and

Production of magnesium salt of bile

Ulf Strenger, 920828

Only process streams are shown. One batch is the basis.



The following **asumption** was made:
(1) Solids content after filtration is 50 %

H_2SO_4 have to be given by PTE or CTA as well as the amount of dried choleic and desoxycholeic acids crude mixture

5 Thyroid powder

For the production of thyroid powder there are the following requirements on the equipment:

(1)	Regeneration system for PE. Similar to acetone regeneration system, but half the size of the vessels and the same distillation column.
(2) 01.7	(a) Connection to PE regeneration system
(3) 03.2	(a) Connection to sewage and ex-proof motor
(4) 03.4	(a) Connection to PE regeneration system
(5) 03.6	(a) 0.4 MPa working pressure because of the use of N_2 (b) Connection to N_2 and PI. (c) Decantation device connected to PE regeneration system. (d) Ex-proof motor
(6) 03.1	(a) Ex-proof motor
(7)	Ex-proof lamps and other electrical installations in room 11 and 16.
(8) 02.6	addition of device for the decantation of water from the bottom waste product in the regeneration column. See the process description for insoluble pancreatin.

The process is described as follows:

(1) 50 kg of frozen thyroid gland is minced in the grinding machine 01.1.

(2) The minced gland is dried in the vacuum drier 01.7, where 35 kg of water is evaporated.

(3) The dried gland's fat is extracted with petroleum ether (PE) in the vessel 03.6. The amount of PE is 60 liters.

(4) After 15 minutes of stirring, the PE is decanted and sent to the regeneration system for PE. The PE is then regenerated in the distillation column. The bottoms will contain water both from the gland and from condensing steam since the column is heated by direct steam. The separation of fat from the waste water should be performed in 02.6 described above. The fat can, if there are facilities, be used in the production of soap. If there are no such facilities, we suggest destruction of the fat by means of burning.

(5) The gland is washed with another 60 liters of PE for one hour.

(6) The suspension is separated in the centrifuge 03.4. The PE is collected as above.

(7) The gland is dried is the vacuum drier 01.7.

(8) The dry gland is heated to 60 °C together with 45 kg of demineralized water in vessel 03.1 for 30 minutes. 1.2 liters of hydrogen peroxide is added and the

temperature increased to 85 °C for 30 minutes.
(9) The suspension is filtered in the filter 03.2.
(10) The solid part is transferred back to the vessel 03.1 and is washed with 22.5 liters of ethanol (95%).
(11) The slurry is then filtered in 03.2.
(12) The solid part is dried in 01.7.
(13) The dried product is 9.7 kg thyroid powder per batch.
Comments: If the water in the purification step could be separated with decantation instead of with filtration the procedure would be facilitated since the solid should be washed in the same vessel as it is extracted. By adding a sight glass to 03.1, this decantation would be possible.

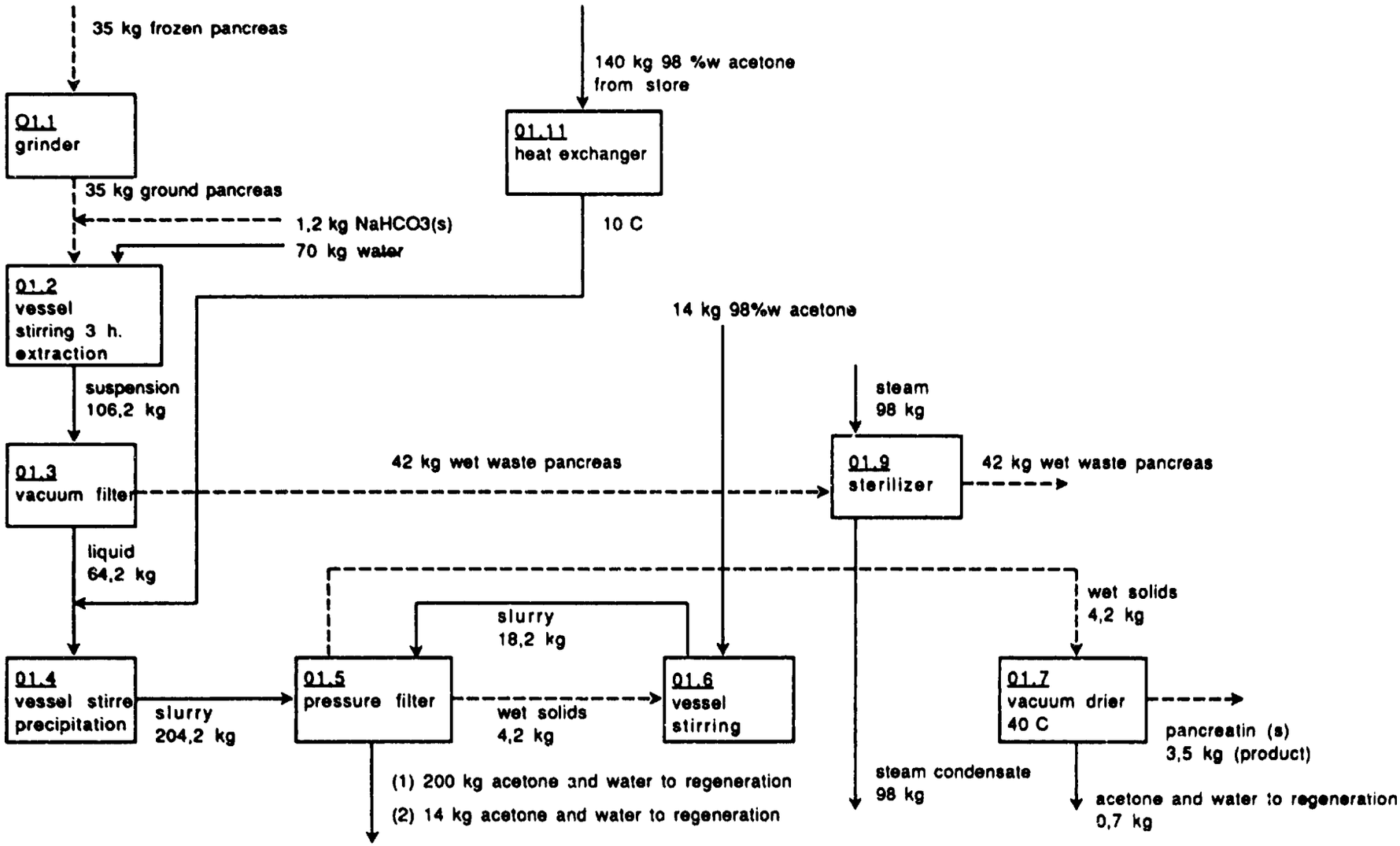
6. Block diagrams

1. Soluble pancreatin
2. Insoluble pancreatin
3. Trypsin and chymotrypsin
4. Magnesium salt of bile
5. Thyroid powder

Production of soluble pancreatin

Ulf Strenger, 920728

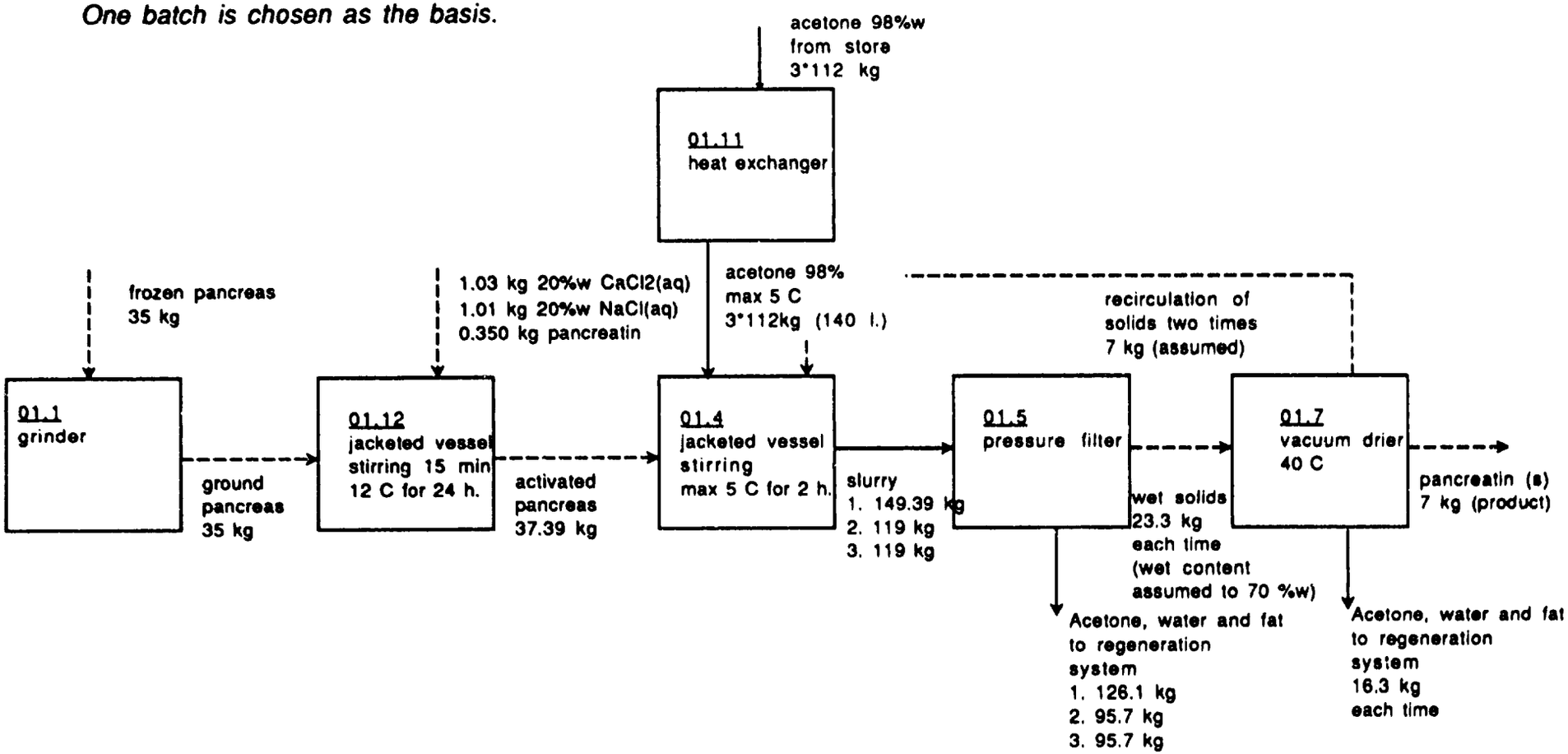
Only process streams are shown. One batch is chosen as the basis.



Production of insoluble pancreatin

Ulf Strenger, 920824

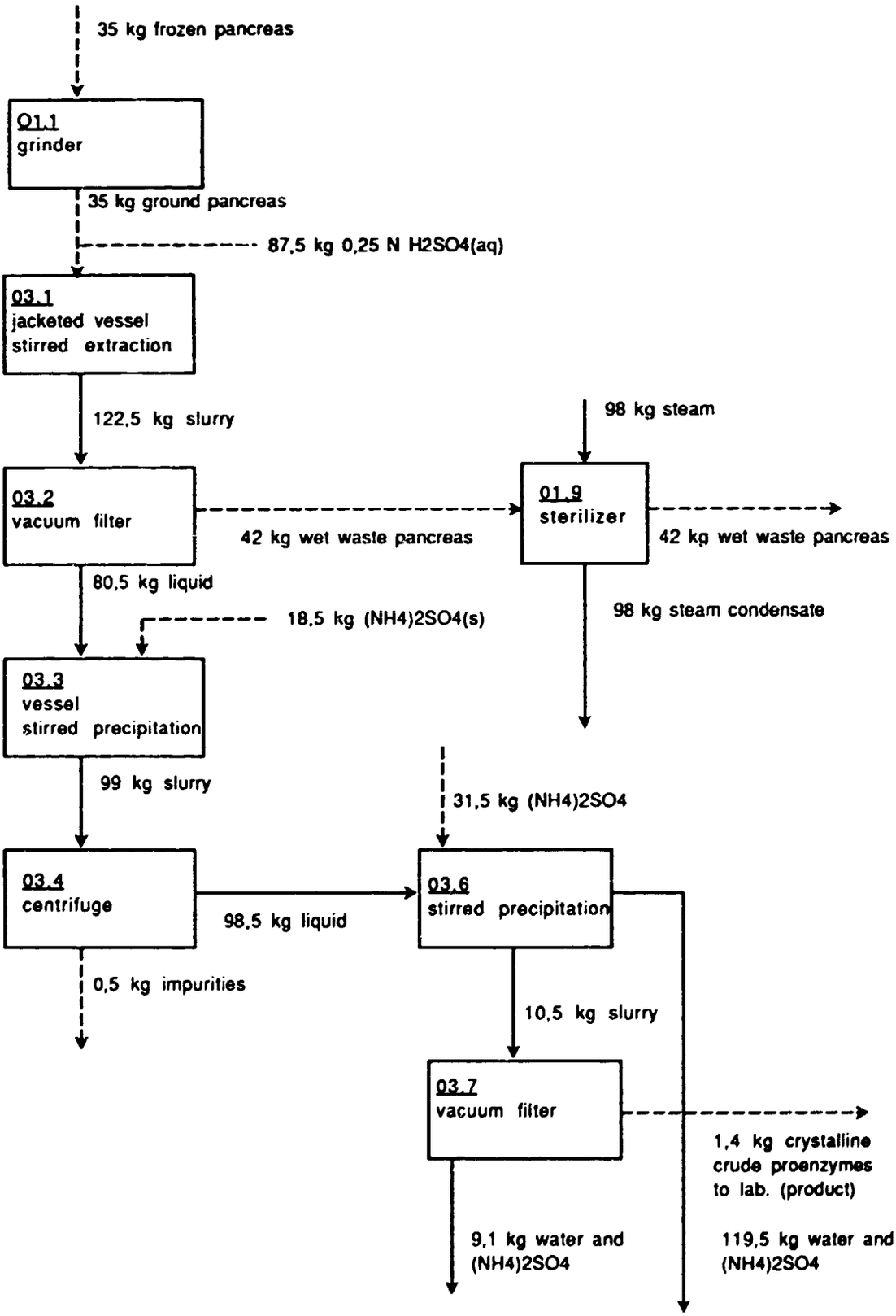
Only process streams are shown.
One batch is chosen as the basis.



Production of chymotrypsin and trypsin

Only process streams are shown.
One batch is the basis.

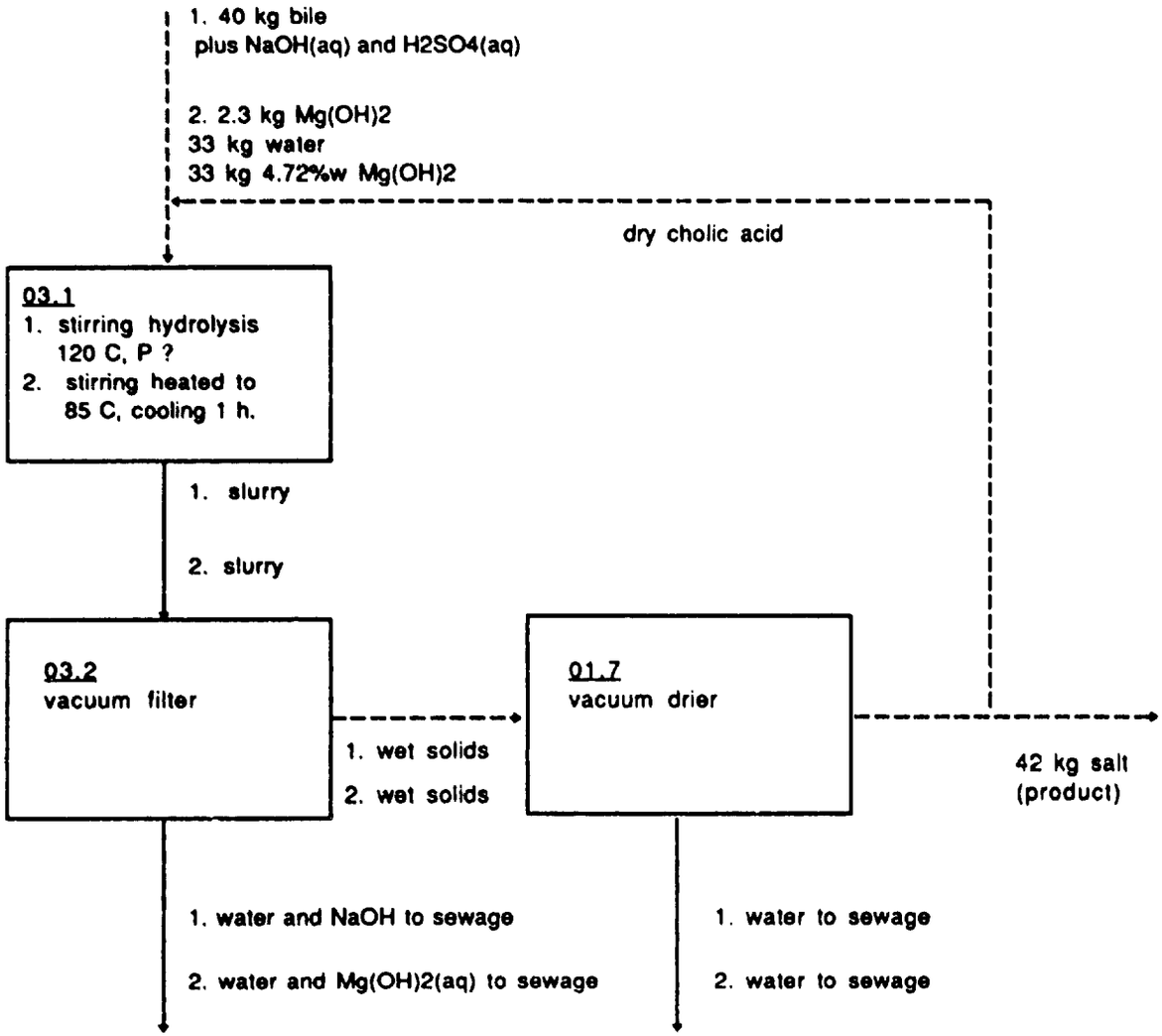
Ulf Strenger, 920728



Production of magnesium salt of bile

Ulf Strenger, 920824

Only process streams are shown. One batch is the basis.

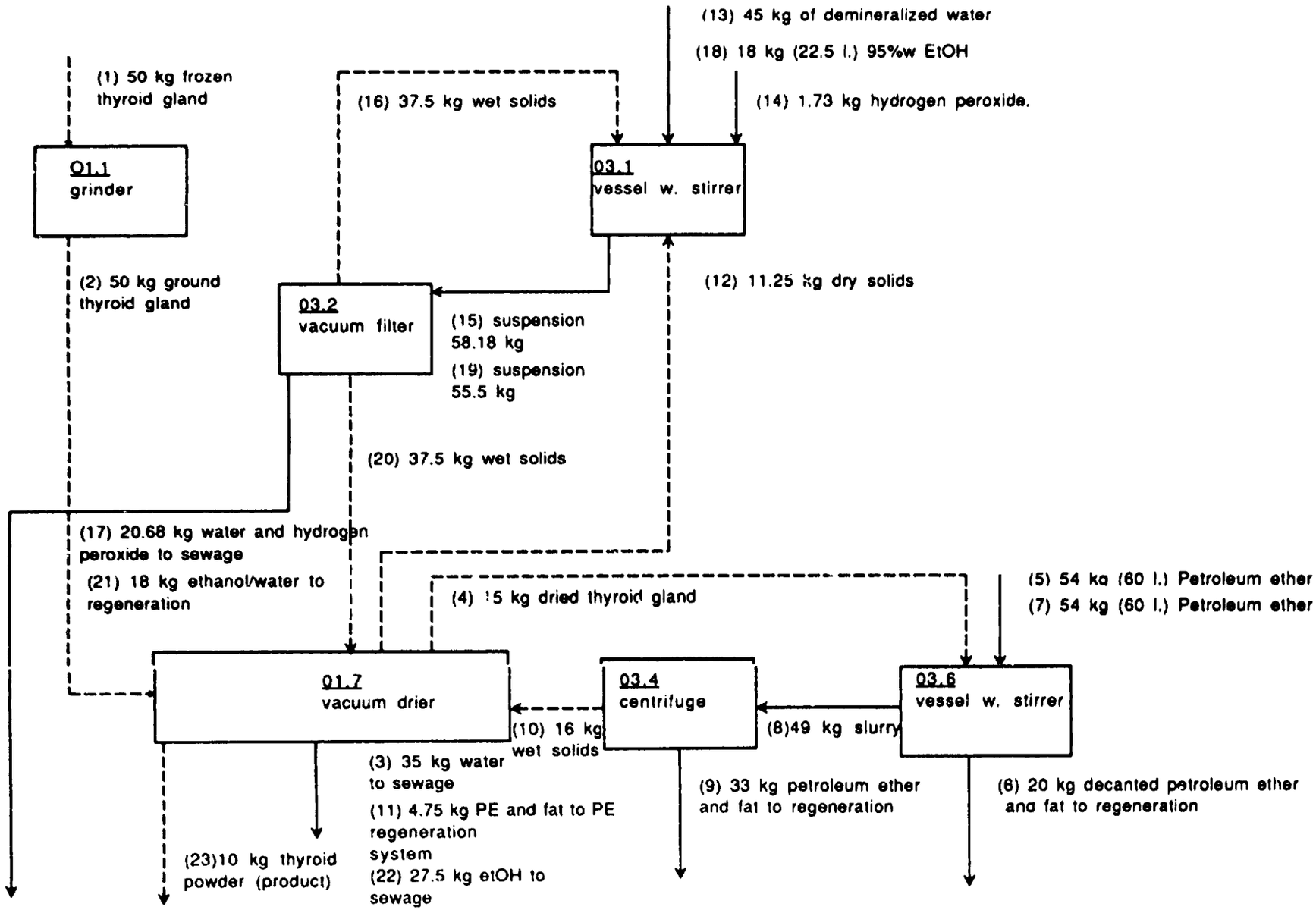


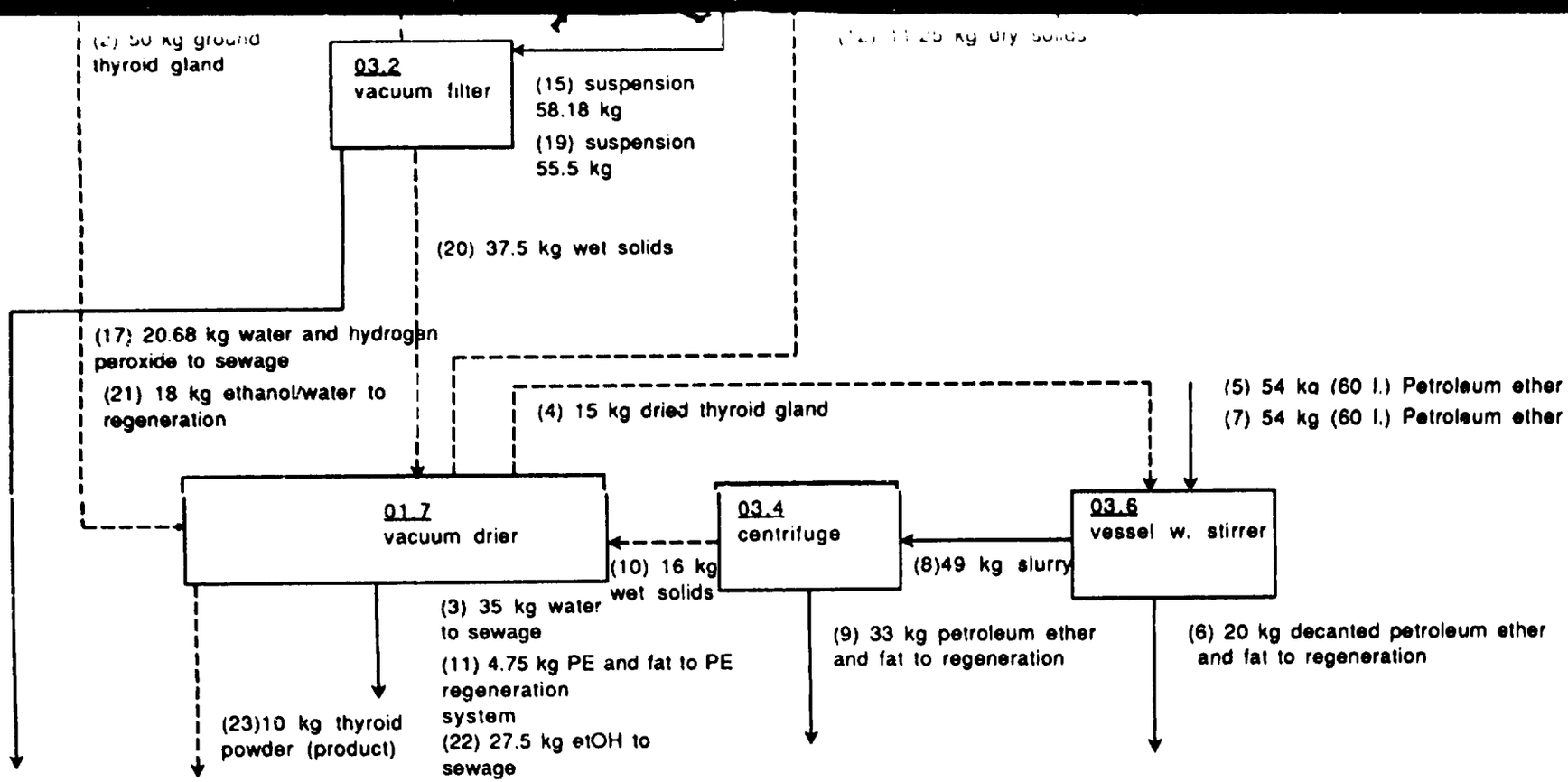
SECTION 1

Production of thyroid powder

Ulf Strenger, 920824

Only process streams are shown. One batch is the basis.
Streams are numbered in chronological order.





In order to complete the material balance the folowing **assumptions** were made:

(1) solids content after filtration is 30% w.
(2) solids content after centrifugation is 70%w.
(3) solids content in gland with fat is 30%.
(4) solids content in gland without fat is 22,5%.
(5) yield of powder is 20%.
(6) the amount og dencanted PE s 20 kg.
(3)-(5) are according to the fellows report.

SECTION 2

RECOMMENDATIONS ON LOCALLY PURCHASED EQUIPMENT AND LAY OUT

Contents

1 General comments about vessels

1. Where downpumping axial flow impellers are used, the ratio between impeller diameter and vessel diameter should be 0.3 to 0.5. The clearance bottom-impeller should be 0.25-0.50 times the diameter of the vessel and baffles should be used. The width of the baffles should be approximately 10 % of the diameter.

Where anchor agitators are used, no baffles are needed. The anchor should be approximately 90-95% of the vessels diameter.

Further design of the stirrers, baffles and motors will be given later. The choice of that equipment will be based on DICI's standards. Optimal stirring will not be promised. The rotational speed of each stirrer remains the same as specified by PTE, except for in some cases that are described below.

2. The motor, gear box, bearings and shafts must be exactly specified on drawings and in quotations. Tenancies of the shafts must be calculated and taken into account.

3. By exactly specified is meant supplier, data sheets and lists of spare parts and prices. The information in the data sheets should be detailed enough so that it is possible for DICI to make detailed design and the operator's manual.

4. All drawings should be equipped with a list, including explanations, of the sockets. The list must be written in both Vietnamese and English. The same should be for all explanations in the drawings.

5. Welds should not be placed in the bends. See illustrations.

6. The pressure vessels have to be recalculated according to some international standard that DICI decides in agreement with UNIDO.

7. The packings have to be placed as described in the illustrations.

8. The outlets of the vessels must be smooth. See illustrations.

9. The shape of the vessels should be smooth. See illustrations.

10. Finish/stroke/polish rough surfaces as welds, to be the same level of finish as of the original stainless steel plate material. The material must be protected so that the quality of the sheets is preserved.

11. If thermometer pockets has to go through a jacket, it should be drawn as in illustration.

2 Locally purchased equipment

The following comments are based on the drawings from Bason Shipyard, but the new requirements arising from the inclusion of new products are also taken into account.

01.2
(a) No baffles should be used.
(b) The socket on the top has to be corrected from 150 to 200 mm according to UNIS' report.
Not used in the production of new products.

01.3
(a) The reinforcement between the legs and the vessel has to be designed and shown on the drawing.
(b) A textile filter medium has to be chosen and purchased.
Not used in the production of new products.

01.4
(a) Baffles have to be designed and drawn.
(b) The diameters of the sockets have to be corrected. The cut L-L is incorrectly drawn and has to be corrected. Weld on the top is incorrectly placed as described in section 1 at point no.4.
(c) The vessel has to be equipped with a jacket (insoluble pancreatin)
(d) jacket connected to cw and cwc (insoluble pancreatin)
(e) TIC for keeping max +5 °C (insoluble pancreatin)
(.) hole on top for feeding (insoluble pancreatin)

01.6

(a) the cut A-A is incorrectly drawn and has to be corrected.
(b) The vessel is unstable as drawn. It has to be stabilized and shown on the drawing.
(c) The volume has to be 50 l.
(d) The power of the motor should be 1.5 kW and give 200 RPM.
(e) All welds has to be shown on the drawing.
(f) The vessel has to be designed for under pressure.
(g) Detail no. I shows the mistake mentioned in section 1 at point no. 4 and has to be corrected.
Not used in the production of new products.

01.7

This item has to be totally recalculated, redesigned and redrawn as a vacuum tray drier heated by hot water (if it is needed for keeping the temperature at 40-60 °C). The temperature of the material that is dried mustn't exceed 60°C.
(a) All connections to utilities and regeneration system, vacuum etc have to be shown.
(b) It must be shown how the heat is distributed inside the drier.
(c) It must be shown how the trays will slide.
(d) Condenser and vacuum pump must be exactly specified.
(e) The control system must be exactly specified.
(f) The body and doors have to be designed for vacuum. Material and the thickness must be specified.
(g) The number of, the material and the thickness of the trays must be specified.
(h) There must be an explanation of how the doors are opened and closed.
(i) Connection to sewage (Magnesium salt of bile).
(j) Connection to PE regeneration system (Thyroid powder).

01.9

(a) It has to be shown how the top is locked and opened. It must be easy to operate. As it is now, the height is 190 cm.
(b) All packings must be drawn and specified.
(c) The basket should not be drawn upside down.
(d) Insulation must be specified on the drawing.
(e) The jacket must be calculated for pressure in according to an international standard.
(f) The thermometer has to be consistent with UNIS and it mustn't go through the jacket.
(g) The outlet mustn't go through the jacket.
(h) Some kind of support in the bottom must be designed
No additions because of the new products.

01.9 Lifting device

(a) This item has to be exactly specified. It is poorly specified in the present drawing, for example the material and the sliding mechanism are not specified.

01.10

This item is canceled.

01.11

This item has to be recalculated, redesigned and redrawn.

(a) The allocation of the streams must be changed, since as it is shown in the drawing, the heat transfer will go in the wrong direction.

(b) Tie rods and spacers have to be designed and used.

(c) International standards has to be respected because of the pressure.

(d) Insulation has to be designed and specified.

(e) All in- and outlets should be moved to the cylinder.

(f) The cylinder is too small to contain 100 tubes, since the pitch should be $1.5*d_{tube}$. This has to be corrected.

(g) All welds must be specified and drawn (see drawing at A-A).

(h) The cut A-A and detail I have to be consistent.

(i) Has to be designed so that it is possible to cool the acetone to + 5°C (insoluble pancreatin).

01.12 (insoluble pancreatin)

This item is new. The requirements are the following:

(a) 40 RPM-stirrer, no baffles, 50 l. stainless steel and jacketed

(b) jacket connected to cw and cwc

(c) TIC for keeping +12°C

(d) light and sight glass for the decantation

(e) hole on top for easy feeding, decantation and emptying of the remaining pancreas.

Points (b) and (c) could be canceled if it was possible to have the autolysis at 4°C, since the vessel then might stand in the cold room if there is enough space.

02.2

(a) In the drawing reference is made to drawing C91-UNDO-V-019, should be --014.

(b) There are insufficient details in the drawing about the level indicator. For safety it has to include valves. An exact specification is required.

(c) On --14, the diameter is 125, but on drawing 120. This have to be corrected.

02.3

(a) This item has to be exactly specified and must follow the requirements of the new products. That means it has to produce water of 0°C.

02.4

(a) In the drawing reference is made to drawing C91-UNDO-V019, should be --015.
(b) The socket has been changed from 25 to 32 mm. This has to be consistent with the way acetone is transported to the factory.
(c) Another socket, detail VI, is 32 mm but has to be changed to 40 mm.
(d) It has to be checked that the fastening mechanism is made at installation.
(e) A-A is canceled.

03.1

(a) In order to fit the supporting steel construction, there have to be four legs, not three as in drawing.
(b) The baffles shown in the drawing are cancelled.
(c) The volume of the vessel should be 200 l. and the heat transfer area has to be used efficiently.
(d) The drawing shows two cooling water inlets, while one is enough.
(e) Details II and IV have to be corrected.
(f) The motor has to be ex-proof. (Thyroid powder.)
(g) jacket connected to steam and steam condensate as well as to cw and cwc. (Magnesium salt of bile.)
(h) TIC to steam - might be manual. (Magnesium salt of bile.)
(i) Connection to N_2 in order to pressurize the vessel. (Magnesium salt of bile.)

03.2

See comments on 01.3+
(a) Connection to sewage.(Magnesium salt of bile and Thyroid powder.)

03.3

(a) Baffles are needed and has to be designed and drawn.
(b) The angel of the inclination of the propeller has to be shown on the drawing.
Not used in the production of new products.

03.5

This item is canceled.

03.6

(a) The sight glasses have to be exactly specified and shown on drawing.
(b) Baffles have to be designed and drawn.
(c) Increase the volume of this vessel by 25%
(d) The motor has to be ex-proof. (Thyroid powder.)
(e) 0.4 MPa working pressure. (Thyroid powder.)
(f) Connection to N_2 and a PI. (Thyroid powder.)
(g) Decantation device connected to PE regeneration system. (Thyroid powder.)

03.8
This item is canceled.

04.1
(a) See 02.2 b.
(b) There have to be reinforcements in the bottom.
(c) The bottom plate has to be designed for ovality in the cylinder. There should be ca: 5 mm extra diameter.

04.3
(a) The vessel should be designed as in PTE's report in june 91 p. 50.
(b) The socket in teh bottom should be 25 mm as in PTE's report in june 91 p. 50.

06.4
(a) A supplier of the temperature indicator must be specified.
(b) Only one detail I should be on drawing.
(c) There has to be a top view of the vessel, in order to see how the sockets are placed.

06.6
(a) This item has to be exactly specified and has to follow international standards.
(b) The position will be horizontal and not vertical as in PTE's report in june 91 p. 21. This will make it easier to maintain.
(c) There should be an intermediate tank for keeping the pressure constant in the system.

09.3
(a) Reinforcements in the bottom have to be designed and shown on drawing.
(b) There is one socket more than needed. It has to be specified or canceled.
(c) See 02.2 b.
(d) On detail VII, the welding is incorrectly placed. See section 1 at point 4. It has to be corrected.
(e) It must be checked that the legs are sufficiently tall, so that it is possible to have a valve, T-connection and a bend at the outlet.

09.4
(a) See 09.3 a,b.
(b) A sight glass on the top cover has to be added according to PTE's report in November 91 p. 46.
(c) Reinforcements of the cylinder should be horizontal, not vertical as in drawing.

05.2 Refrigerated lorry
This item has to be exactly specified.

05.3 Cold room
This item has to be exactly specified.

3 Equipment flowsheets

The following recommendations are based on PTE's report in Nov-91 pp. 40-46 and on the requirements arising from the inclusion of new products.

Production of pancreatin etc. 01
(a) Acetone distribution is from 02.4b, not from 09.3c as written.
(b) Diluted acetone will go to 02.4c, not to 09.3 as written
(c) 01.4 as to be connected to N2. There should not be a cross joint as drawn.
(d) There has to be a valve on the pipe to 01.6.
(e) The number of sockets on 01.5 is only two. There cannot be vent and PI. The outer diameter should be shown instead on the inner.
(f) 01.7 has probably to be connected to hot water.
(g) 01.10 is canceled.
(h) The jacket on 01.4 connected to cw and cwc (insoluble pancreatin).
(i) 01.7 connected to sewage (Magnesium salt of bile) and to PE regeneration system (Thyroid powder).
(j) 01.12 jacket connected to cw and cwc and some device for decantation (insoluble pancreatin).

Store for inflammable liquids 02
(a) It has to be checked if the feeding pipe of fresh acetone is in accordance with the means of transportation of acetone to the factory.

Safety
All equipment and pipes were acetone/PE/ethanol is used have to be connected to earth. This have to be shown on drawings.

Unit for regeneration of acetone 02
(a) Acetone for regeneration is not from 09.3 as written, but from the pump 2.5b.
(b) No warm water distribution is needed except for cleaning purposes. There should be a nozzle.
(c) All equipment in the drawing has to be specified.
(d) All distribution lines have to be labeled.

Unit for regeneration of petroleum ether 02 (new-Thyroid powder)
The same as the acetone regeneration system. The same column should be used but new vessels for storage. The same design as in for acetone can be used, but with half the size.
A decanter 02.6 should be installed as described in the process descripion of thyroid powder.

Unit for regeneration of acetone 02(new- insoluble pancreatin)
A decanter 02.6 should be installed as described in the process descripion insoluble pancreatin.

Production of chymotrypsin and trypsin etc. 03
(a) Items 03.5 and 03.8 are canceled.
(b) There has to be a pump between 03.4 and 03.6.
(c) A supplier of the siphon has to be specified.
(d) Jacket on 03.1 connected to steam and steam condensate as well as to cw and cwc. (Magnesium salt of bile.)
(e) 03.1 connected to N_2 in order to pressurize the vessel. (Magnesium salt of bile.)
(f) 03.2 connected to sewage.(Magnesium salt of bile and Thyroid powder.)
(g) 03.6 connected to N_2 and a PI. (Thyroid powder.)
(h) 03.6's decantation device connected to PE regeneration system. (Thyroid powder.)
(i) 03.4 has to be connected to the PE regeneration system. (Thyroid powder.)

Production of dry bile 04
(a) There should be a 25 mm pipe from 04.3 to sewage. the socket has to be changed.

Unit for central compressed air and central vacuum
(a) The vessel in 06.1 is 350 l. according to the invoice (Labortechnic), not 250 l. as in drawing. Has to be corrected.
(b) According to the invoice, the air drier is not a silica gel drier as in the drawing , but a refrigerating drier.

Production of distilled water and demineralized water
(a) There should be written that the demi water distribution is only for lab.
(b) It has to be checked whether the tap water has the pressure of 250 kPa as written in the drawing.
(c) The inlet to 09.1 should be specified - tap water. it should be written in the drawing that 09.1 is a water distiller.

Cooling water system

The freezing point of the cooling system has to be lower than 0°C. The agent that is used to perform this mustn't be aggresive to the steel pipes. Ethylen glycol is suggested.

Distribution of utilities

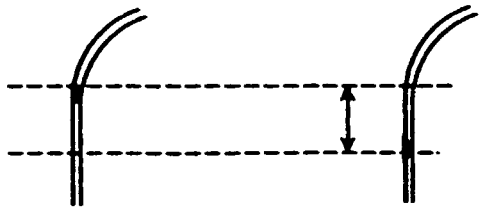
The different sizes of the pipes in different rooms reflect the consumptions in each room. Therefore the recommendations of PTE should be followed. The vacuum pipe in 09 should be 50 mm as in the other rooms. Demineralized water should be used in the lab and in the steam generator.

Drainage

Drainage has to be installed in all rooms where production takes place as well as in the labs.

4. ILLUSTRATIONS

1.



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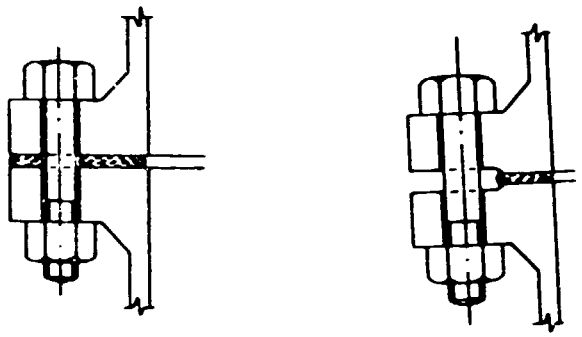

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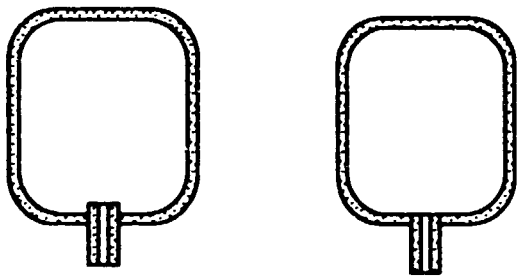

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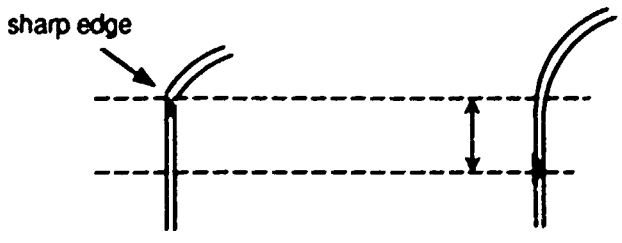

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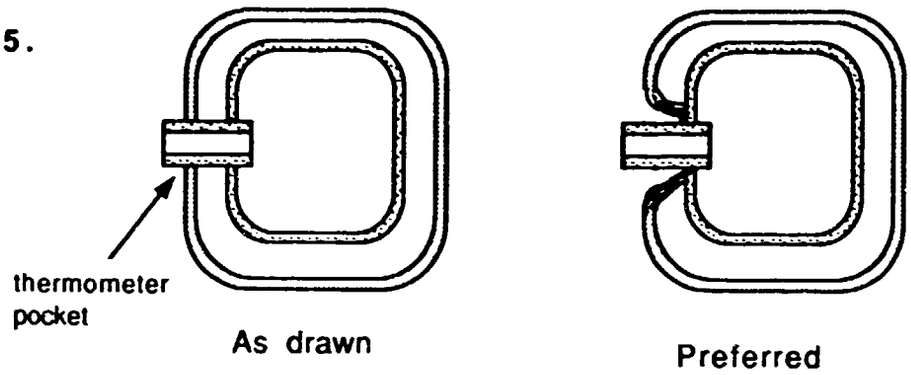

As drawn

Preferred

LITERATURE STUDY

Contents

1 Conclusions

The proposed methods for the production of pancreatin (two methods) and of chymotrypsin and trypsin (one method) follows the general procedure for the production of industrial enzymes from animal organs. There are some differences though. They are (1) no activation step and no defatting step in the production of soluble pancreatin and of chymotrypsin and trypsin, (2) no milling of the product.

A literature search resulted in five alternative methods (methods no.1-5, described below) for the production of pancreatin and of trypsin. All methods are different, but show similarities. The proposed procedures for soluble pancreatin and for trypsin/chymotrypsin are most similar to method no. 2 and the proposed method for insoluble pancreatin is most similar to method no. 1.

The yields and activities of the products are given in different units and are not easily compared, why no comments about which method is the best are given.

2 Production of industrial enzymes from animal organs

The general sequence of steps in the isolation of enzymes is described below based on McKetta and Kirk-Othmer. The fundamental process for producing enzymes is similar, regardless of enzyme source.

Grinding of the frozen organs, freed from fat and connective tissue before freezing, with machines generally used in the meat industry. Besides mechanical grinding, enzymatic digestion by indigenous enzymes and autolysis, can also be employed. Fat attached to the organs interferes with subsequent purification steps and can be removed with organic solvents, which might , though, decrease the enzymatic activity of the product.
Extraction of the enzymes with a buffer solution.

Filtration or centrifugation in simple equipment of the residual organ matter.
Concentration can be performed by means of thermal methods, precipitation or membrane filtration. Precipitation is the simplest procedure for concentration of enzymes. By changing the enzymes' environment they can be made to agglomerate. This can be realized by salts, organic solvents, polymers and by elaborating the pH.
Ammonium sulfate is commonly used in concentrations ranging from 20 to 80 % saturation. The optimal concentration is determined from experiments. Acetone is also commonly used, but the concentration of solvent and the temperature (0-10°C) have to be carefully controlled.
Filtration or centifugation of the precipitate.
Drying in vacuum at low temperature (20-50°C).
Purification: For many industrial applications, partially purified enzyme preparations will suffice; however, enzymes for analytical purposes and for medical use must be highly purified. Special procedures employed for enzyme purification are crystallization, electrophoresis, and chromatography. Salting out of enzymes can be performed from ammonium sulfate solution. In general, salting out doesn't give sufficient purity.

3 Production of enzymes from pancreas

The descriptions below are directly cited from the references. One example from each patent is chosen.

Method I (Chang C.T. et al., 1985)
Hog pancreas weighing 500 g was minced into pieces while still frozen, and homogenized in a Waring blender with one of the following media, namely, 500 ml water or 500 ml of 0.25% calcium chloride solution or 50 g of minced hog duodenum suspended in 500 ml of water. After removing the adhesive substances by filtration through nylon cloths, the mixture was allowed to stand at 4°C for autolysis for one to four days, and then 500 ml of acetone was added to the mixture by stirring. The precipitate was collected by centrifugation at 8000 g for 20 minutes. The collected precipitate was rehomogenized with 500 ml of acetone in the blender, and the mixture was centrifugated at 8000 g for 10 minutes and the supernatant solution decanted. This procedure was repeated once again with 500 ml af acetone-ether (1:1) and once again with 500 ml of acetone-ether (1:2). The almost dried residue was ground in a mortar under ventilated hood to a dry powder and then stored in a desiccator until use.

From the results, it may be concluded that a pancreatin containing rather high activities of three digestive enzymes could be prepared by autolyzing the pancreatic homogenate in water without any addition or with calcium chloride at 4°C for one to two days.

The specific activity (units/mg solid) was 22.1 for lipase (measured by the hydrosis

of Tween-20), 48.5 for α-amylase (measured by the hydrosis of starch) and 183 for protease (measured by the hydrosis of casein), when the pancreas were autolysed with calcium chloride solution at 4°C for one day.

Method 2 (Madhusudhan K.T. et al., 1988)

This is a method for preparation of chicken pancreatic enzymes for clinical use.

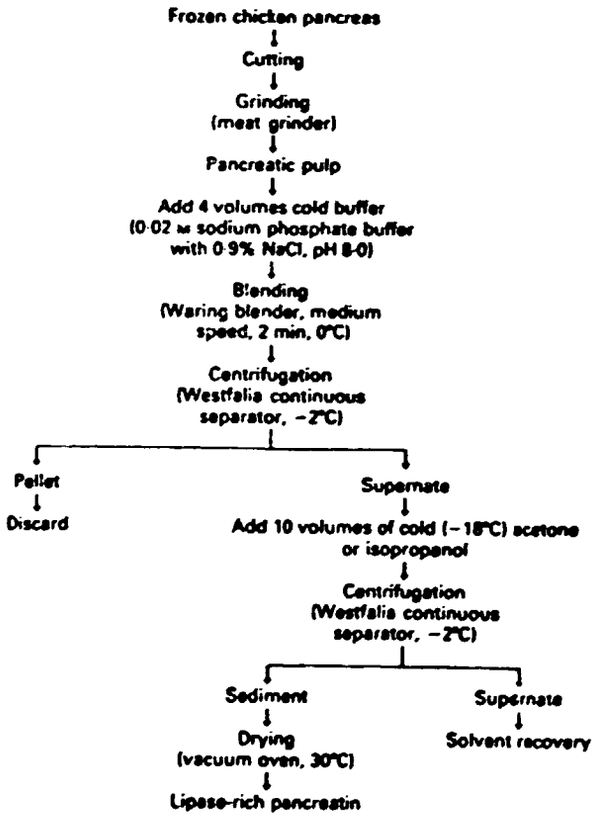

Figure 1. Flow diagram for the preparation of lipase-rich pancreatin.

The specific activity (units/mg solid) was 67.3 for lipase in laboratory scale and 10.4 in pilot plant scale when pancreatin was prepared by acetone precipitation. The specific activity (units/mg solid) was 29 for lipase in laboratory scale and 24 in pilot plant scale when pancreatin was prepared by isopropanol precipitation. The difference in lipase activity between laboratory scale and pilot plant scale is believed to be a result from less

effective mixing. The lipase activity is was expressed as micromoles of fatty acids liberated per minute.

Method 3 (Lewis, S.H., 1976)

To 318 kg of hashed pork pancreas were added 2.275 kg (0.716 %w) of $CaSO_4.2H_2O$ slurried in 17.5 liters of deionized water. After the calcium sulfate slurry was thoroughly mixed into the pancreas, 31.8 kg of hashed pork duodenum were added and thoroughly mixed therein. Finally 66.3 liters of acetone (C.P. grade) were thoroughly combined with the mixture. The mixture was then stored for 7 days at a temperature of 4 °C in order to complete activation. Thereupon, the pancreatin was defatted by washing the wet, fatty mass with sufficient number of washes of room-temperature acetone to dehydrate and degrease the tissue. The acetone-wet tissue was then heated under vacuum until the temperature of the material reached 82 °C. The vacuum was then released and the temperature of the material maintained at 82 °C under atmospheric pressure for three hours. The material was exposed to the atmosphere during this step because the moisture in the air promotes the destruction of Salmonella bacteria. Thereafter, the vacuum drying oven was cooled to 49°C and maintained at that temperature under vacuum for one hour. Thereupon the dried pancreas was removed from the dryer and collected for the particle comminution or milling operation.

Method 4 (Fabian, F., 1973)

100 gms minced pancreas are mixed with 80m mls. water and warmed to 25 °C. Then 20 mls normal ammonium hydroxide are added thereto and autolysis is continued for 30 minutes. The mixture is then treated with three succesive volumes of 750 mls, 350 mls, and 250 mls of n-butyl or iso-butyl alcohol. At this stage the pancreatin is defatted and the butyl alcohol is decanted from the partially settled sludge. This is slurried with two volumes of water which can readily be centrifuged, giving a residue of only slight enzyme activity and a somewhat cloudy solution. To this solution 9 volumes of ethyl alcohol are added. A precipitate of high activity pancreatin is obtained, the supernatant solution containing inactive degradation products of the original pancreas, but also some unprecipitated enzymes.

The activity of this pancreatin is more than 3*NF.

Method 5 (Leidholdt, F., 1972)

Inhibitor-free readily water-soluble enzymes are prepared from comminuting animal pancreas which have been frozen immediately after slaughtering, extraction said

pancreas with an alcohol containing 1-2 carbon atoms, treating said extract with a water-insoluble liquid solvent selected from the group consisting of a butyl alcohohol and an ether, precipitating said enzyme from the resulting clear layer consisting of an alcohol containing 1-2 carbon atoms and a ketone at a temperature below 10 °C and recovering the enzyme. An example of the invention follows:

For preparing an inhibitor free, readily water-soluble trypsin, 50 kg pancreas glands from pigs were frozen at - 15°C immediately after slaughtering. After a freezing time of 24 hours, this material was passed in frozen condition through a comminuting device, e.g. a mincing machine having perforated disc with holes of 3-5 mm in diameter. To the comminuted pancreas material placed in a container there were added 50 kg 95 ethyl alcohol and 150 liters of tap water; the mixture was slowly stirred for 30 minutes avoiding formation of an emulsion and then allowed to stand in the covered container for 12 to 13 hours at room temperature.

Experiments with other solvents such as isopropyl alcohol gave less favorable results since the protein structure of the enzyme and therewith its activity are reduced.

After an aging time of at least 12 hours, 5 kg of kieselguhr (diatomaceous earth) are added to the mixture which is slowly stirred whereby the pancreas fibers assume after a short time rope-like appearance. When this stage is reached, the whole batch is passed through a fine sieve to separate the pancreas fibers material from the turbid pancreas juice. The pancreas fiber material can be further squeezed of a filter press and the resulting press juice may be combined with the turbid pancreas juice. About 130 liters of pancreas juice and, based of the dry substance, 20 kg of pancreas fiber material are obtained. The pancreas juice is made up to 200 liters of liquid by addition of water and mixed with 15 parts by weight of butyl alcohol per 100 parts by weight of juice; the mixture is thoroughly stirred for about 5 minutes and then allowed to stand for 12 hours at room temperature. Upon standing a clear layer and a turbid layer containing substantially fiber residues and foreign proteins are formed. The clear layer is carefully removed by suction, mixed with 1.15 liters of ethyl alcohol per liter of clear solution, and then aged for 6 hours at +5 °C during which time precipitation of trypsin inhibitor-free trypsin occurs. After an aging time of about 6 hours, the batch is filtered through a suction filter or a Buchner funnel whereby a total of 650 g (dry basis) purest, readily water-soluble and inhibitor free trypsin is obtained.

An analysis of this substance showed that this trypsin contained about 450 to 500 Wilstätter units per g substance.

The pancreas fiber material obtained after extraction with ethyl alcohol and squeezing on the filter press is stirred with acetone in a weight ratio of 1:5 and allowed to stand for about 10 minutes. After this time, the mixture is passed through a filter or a sieve and the residue is air dried and ground. This way, about 6 kg pure pancreatin are obtained containing per g of substance 20 units lipase, 40 units trypsin and 60 units chymotrypsin (Wilstätter method).

4 Literature search

Source	year	keywords
Chemical Abstracts	1967- 1992	Pancreatin Thyroid powder Magnesium salt of bile Biliar salts - production of, - manufacture of

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