MELiSSA

TECHNICAL NOTE

TECHNICAL NOTE 80.241

Recommended design and integration strategy
Detailed design Waste Preparation Unit and Compartment I

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1. Introduction

As a follow-up on the compiled TN80.21-22-23 focussing on the different possible options for all the fermentors, materials, instruments, components,… , this TN describes the recommended design for the BELISSIMA loop.

The MELiSSA loop consists of several compartments, all having a dedicated function. The different process conditions and the intermediate separation techniques are expected to eliminate the major part of the contaminants entering the loop.
Within the BELISSIMA contract, a small-scale MELiSSA loop will be constructed to monitor the behaviour of various microcompounds in this model closed loop system. Possible study items include genetic stability and axenicity, minerals, hormones, pharmaceutical drugs, other biosafety issues and countermeasures for microcompounds. All these studies cannot be performed within the current contract but the loop design has to be such that it accommodates for all these investigations on the long term.
The BELISSIMA loop will consist of compartments I to IVa. Although this is not foreseen in the first phases of the contract, the loop should at a later stage be extendible with a higher plant compartment of appropriate size to allow for statistically relevant analysis on the cultivated vegetables.

This document starts with a detailed description of the final loop sizing. It then summarizes the various scenarios which were studied to come to the recommended design. This includes contacts with a selection of suppliers. Finally, this document provides the detailed design for the waste preparation unit and for compartment I. The detailed design of the other compartments forms part of later contracts.

2. Final loop sizing

UBP used the following approach to perform the sizing of the BELISSIMA loop:

- Phase 1: the BELISSIMA flow-sheet calculations are performed on the basis of a CII photobioreactor with a diameter of 19 cm and for two different volumes. It was decided that the final BELISSIMA loop design should provide the flexibility to operate compartment II at a volume of 5 l for periods in which required sampling volumes are low and at a volume of 10 l for periods in which they are high (see also 4). As a result of Phase 1 a simple flow sheet is provided together with a detailed file obtained from the overall mass balance simulator.
- Phase 2: additional simplified flow sheets are produced taking into account a daily sample of 0.5 L after each compartment. Because the sampling cannot be included in the overall simulator, only a simple flow sheet is provided, the main compositions being deduced by mass balances according to the previous step calculation.

The following assumptions were made:

- the feed composition corresponds to the mission scenario detailed in TN80.16.
- the conversion efficiency for CI is unknown since the mission scenario in BELISSIMA differs from the one used in MELiSSA. The results obtained in the contract Engineering of the Waste Compartment can therefore not be transferred to BELISSIMA. For the sizing of the BELISSIMA loop, a conversion efficiency of 50% was used.
- CIVa is sized considering a (quasi) total exhaustion of nitrates in the liquid phase and with a diameter of 19 cm.
- sampling volumes amount to 0.5 l after each compartment. Sampling of the CI effluent occurs on the undiluted stream.

Rates used to estimate the liquid volumes in the global simulator were the following:

- the eliminated organic matter load in CI is 0,0021 kg/l.d
- biomass productivity in CII is 0,0021 kg/l.d
- ammonium load in CIII is 0,0016 kg/l.d
- biomass productivity in CIVa is 0,0014 kg/l.d.

The major constraint of the loop is the functioning of CII. Table 1 summarizes the simulations for a 5 l and 10 l photobioreactor. These were used as a basis for all the calculations and sizing of the BELISSIMA loop.

Figure 1 to Figure 4 show the general flowsheets of the BELISSIMA loop for a 5 l working volume of CII (without and with sampling) and for a 10 l working volume (without and with sampling) respectively. As indicated, the dissolved C is insufficient for CIVa and additional C will have to be supplied through the gas phase. Table 2 to Table 5 show the corresponding detailed mass balances obtained with the global simulator.

The recommended design is based on the scenario with a 10 l working volume for CII and sampling after each compartment. This implies maximal working volumes of 19 l, 10 l, 1 l and 11 l for compartments I to IVa.

The BELISSIMA loop may at some point include a higher plant compartment. In this case, the complete flow from compartment III will be diverted to compartment IVb instead of IVa. The available flow is 10.4 l/d and the nitrate concentration 0.15 g/l (see Figure 4). Data from the University of Guelph indicate that lettuce grows at consumption rates of 0.007 mol nitrate/m².d and 0.005 mol ammonium/m².d, assuming external CO_2 supply. For the BELISSIMA scenario, this would correspond to 2 m², which is acceptable.

Table 1: Simulations of CII functioning for a diameter of 19 cm and assuming 50% conversion efficiency in CI.

	SARTORIUS BBI V = 5 L	SARTORIUS BBI V = 10 L
Maximum performance of the PBR at q_0 = 300-400 W/m^2 (g_{VFA}/d)	11	22
Corresponding input mass flow rate (feed) in the loop (*assumption 50% efficiency for fibers in CI***) (g_{TOT}/d)**	**23 (1/18 man)**	**47 (1/9 man)**
!! All the following data are for nominal light flux at 75-100 W/m^2 !!		
Nominal performance of the PBR at q_0 = 75-100 W/m^2 (g_{VFA}/d)	7	14
Corresponding input mass flow rate (feed) in the loop (*assumption 50% efficiency for fibers in CI***) (g_{TOT}/d)**	**15 (1/28 man)**	**30 (1/14 man)**
Nominal residence time in the PBR (h)	21	21
Nominal biomass concentration in the ouput of CII (g/L)	1.1	1.1
Nominal VFA concentration in the input of CII (g/L)	1.25	1.25
Nominal liquid flow rate (L/d)	5.7	11.4
Nominal resulting concentration of the feed (g/L)	2.6	2.6
Minimum residence time in the PBR (h)	9	9
Minimum biomass concentration in the ouput of CII (g/L)	0.45	0.45
Minimum VFA concentration in the input of CII (g/L)	0.5	0.5
Maximum liquid flow rate (L/d)	13.5	27
Minimum resulting concentration of the feed (g/L)	1.1	1.1
Maximum residence time in the PBR (h)	34	34
Maximum biomass concentration in the ouput of CII (g/L)	1.8	1.8
Maximum VFA concentration in the input of CII (g/L)	2	2
Minimum liquid flow rate (L/d)	3.5	7
Maximum resulting concentration of the feed (g/L)	4.3	4.3

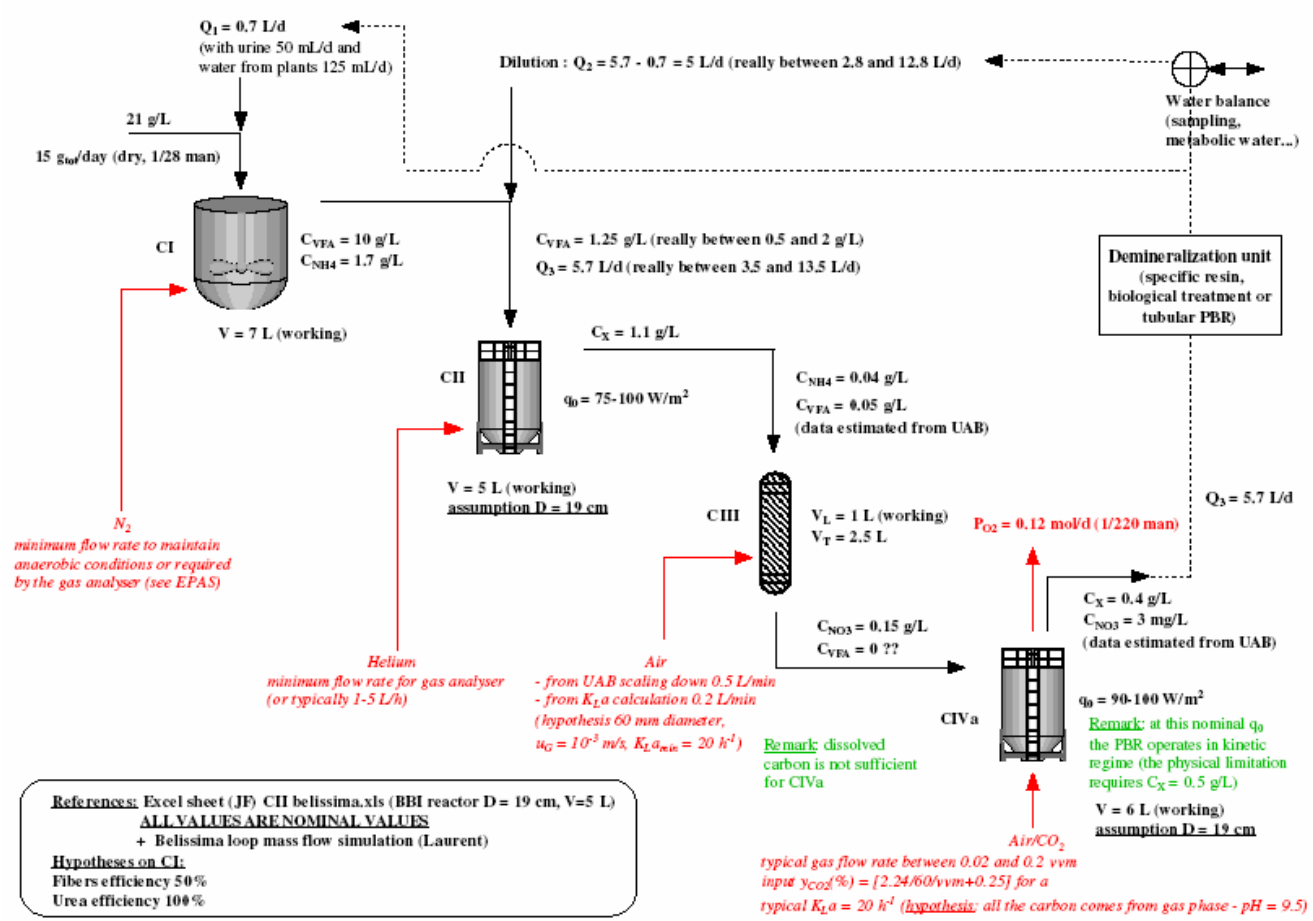


Figure 1. General flowsheet of the BELISSIMA loop for a 5 l volume of CII excluding sampling.

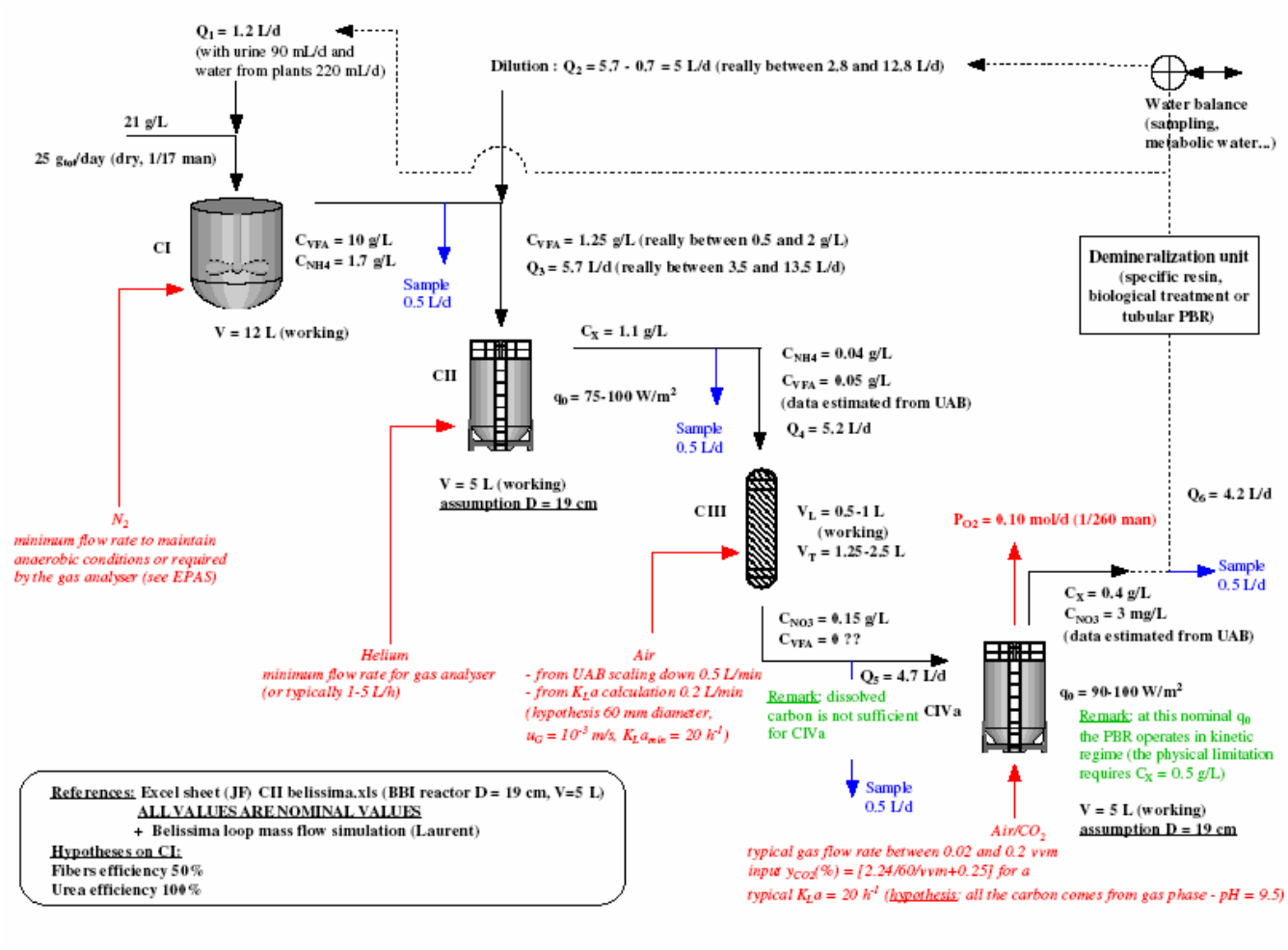


Figure 2. General flowsheet of the BELISSIMA loop for a 5 l volume of CII and including 0.5 l/d sampling after each compartment.

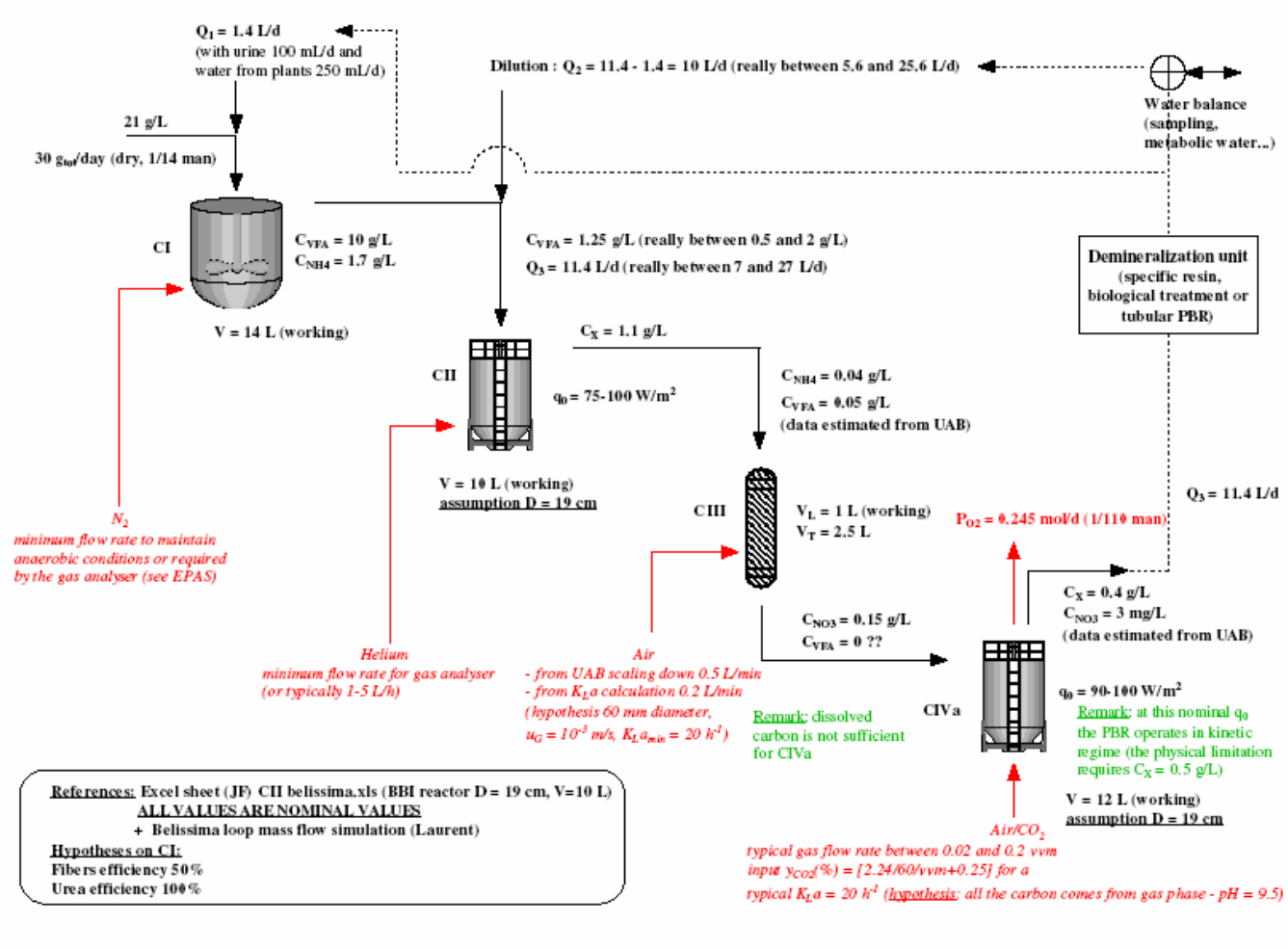


Figure 3. General flowsheet of the BELISSIMA loop for a 10 l volume of CII excluding sampling.

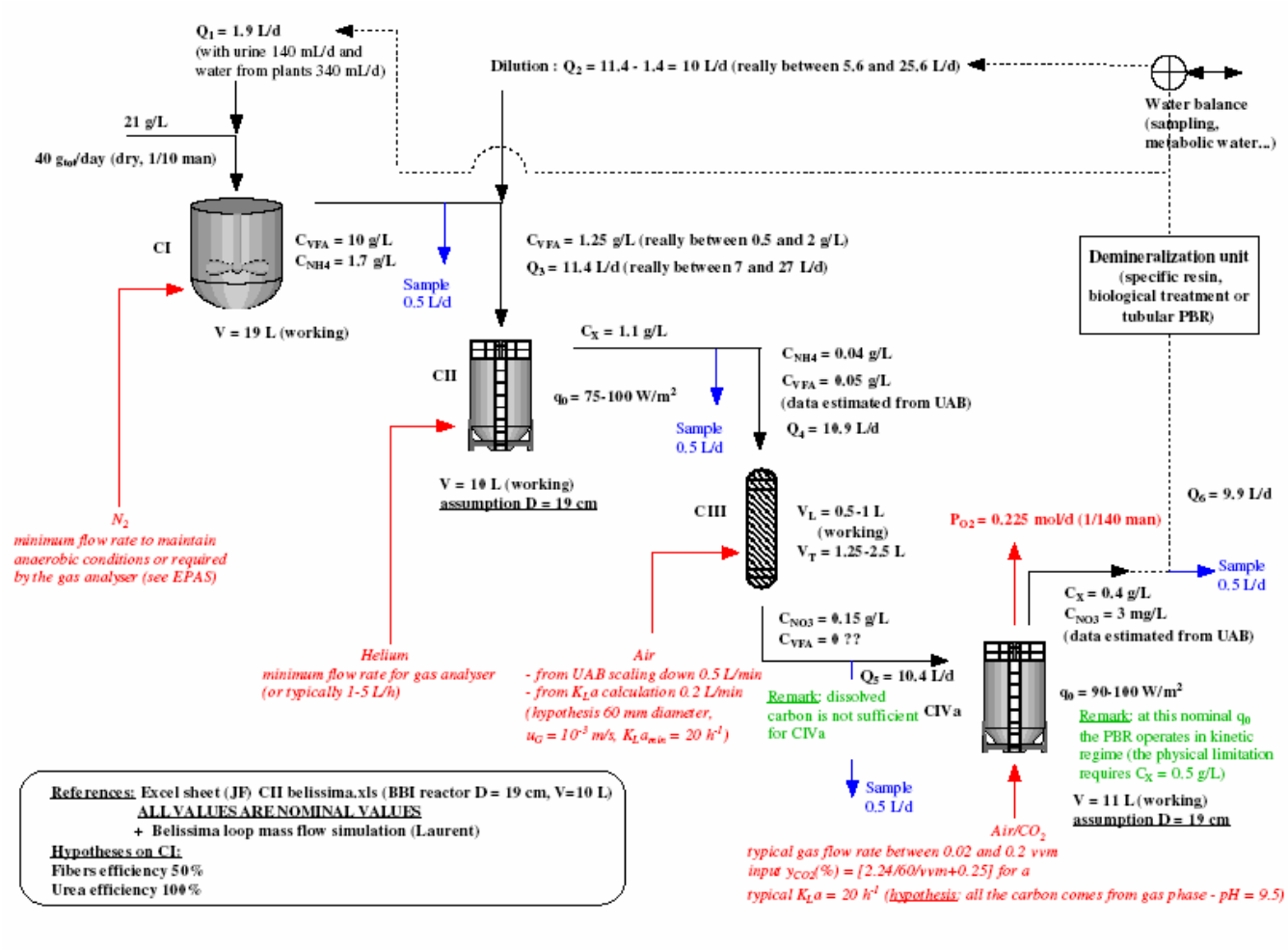


Figure 4. General flowsheet of the BELISSIMA loop for a 10 l volume of CII and including 0.5 l/d sampling after each compartment.

Table 2: Detailed mass balances for a 5 l working volume of CII excluding sampling.

C1	Working Volume	7	L			
	Theoretical OM Load	2.1	g/L.d	Theoretical HRT	7 - 15	days
	Simulation OM Load	2.08	g/Ld	Simulation HRT	9.70	days
	VFA [C¶]	9.46	g/L	Liq Flow rate (after sampling)	0.722	L /day
lobal	degradation efficiency	56%				
C2	Working volume	**5**	**L**			
	Reactor productivity	5.20E-02	g/L/h	[JF Cornet - October 2007]		
	Theoretical VFA load			Theoretical HRT		
	Simulation VFA Load	1.193	g/l	Simulation HRT	0.873	days
	VFA removal efficiency	100%		Liq Flow rate (after sampling)	5.725	L/day
	Biomass [C¶]	1.03	g/L			
C3	Working Volume	1	L			
	Theoretical N load	1.6	g/L/d (maximun ?)	Theoretical HRT		
	Simulation N Load	0.223	g/L/d	Simulation HRT	0.175	days
	nitrification efficiency	98%		Liq Flow rate (after sampling)	5.719	L/day
	Biomass [C¶] -----		g/L			
C4	Working Volume	8	L			
	Reactor productivity	1.53E-02	g/L/h	[JF Cornet - October 2007]		
	Co2 removal efficiency	LIMITING		Theoretical HRT		
	HNO3 remova	48%		Simulation HRT	1.399	days
	Biomass [C¶]	0.17	g/L	Liq Flow rate (after sampling)	5. 719	L/day

Table 3: Detailed mass balances for a 5 l working volume of CII including 0.5 l/d sampling after each compartment.

C1	Working Volume	**12**	**L**			
	Theoretical OM Load	2.1	g/L.d	Theoretical HRT	7 - 15	days
	Simulation OM Load	2.00	g/Ld	Simulation HRT	10.14	days
	VFA [C¶]	9.51	g/L	Liq Flow rate (after sampling)	0.685	L/d ay
lobal	degradation efficiency	56%				
C2	Working volume	**5**	**L**			
	Reactor productivity	5.20E-02	g/L/h	[JF Cornet - October 2007]		
	Theoretical VFA load			Theoretical HRT		
	Simulation VFA Load	1.145		Simulation HRT	0.962	days
	VFA removal efficiency	100%		Liq Flow rate (after sampling)	5.1948	L/day
	Biomass [C¶]	0.99	g/L			
C3	Working Volume	1	L			
	Theoretical N load	1.6	g/L/d (maximum?)	Theoretical HRT		
	Simulation N Load	0.194	g/L/d	Simulation HRT	0.193	days
	nitrification efficiency	98%		Liq Flow rate (after sampling)	4.6889	L/day
	Biomass [C¶] -----		g/L			
C4	Working Volume	8	L			
	Reactor productivity	1.53E-02	g/L/h	[JF Cornet - October 2007]		
	Co2 removal efficiency	LIMITING		Theoretical HRT		
	HNO3 remova	84%		Simulation HRT	1.706	days
	Biomass [C¶]	0.28	g/L	Liq Flow rate (after sampling)	4.18 8	L/day

Table 4: Detailed mass balances for a 10 l working volume of CII excluding sampling.

C1	Working Volume	14	L			
	Theoretical OM Load	2.1	g/L.d	Theoretical HRT	7 - 15	days
	Simulation OM Load	2.08	g/Ld	Simulation HRT	10.42	days
	VFA [C]	10.10	g/L	Liq Flow rate (after sampling)	1.344	L/day
Global	degradation efficiency	57%				
C2	Working volume	10	L			
	Reactor productivity	5.20E-02	g/L/h	[JF Cornet - October 2007]		
	Theoretical VFA load			Theoretical HRT		
	Simulation VFA Load	1.196		Simulation HRT	0.881	days
	VFA removal efficiency	100%		Liq Flow rate (after sampling)	11.35	L/day
	Biomass [C]	1.03	g/L			
C3	Working Volume	1	L			
	Theoretical N load	1.6	g/L/d (maximum?)	Theoretical HRT		
	Simulation N Load	0.437	g/L/d	Simulation HRT	0.088	days
	nitrification efficiency	98%		Liq Flow rate (after sampling)	11.34	L/day
	Biomass [C] -----		g/L			
C4	Working Volume	8	L			
	Reactor productivity	1.53E-02	g/L/h	[JF Cornet - October 2007]		
	Co2 removal efficiency	LIMITING		Theoretical HRT		
	HNO3 removal	49%		Simulation HRT	0.705	days
	Biomass [C]	0.17	g/L	Liq Flow rate (after sampling)	11 .34	L/day

Table 5: Detailed mass balances for a 10 l working volume of CII including 0.5 l/d sampling after each compartment.

C1	**Working Volume**	**19**	**L**			
	Theoretical OM Load	2.1	g/L.d	Theoretical HRT	7 - 15	days
	Simulation OM Load	2.15	g/Ld	Simulation HRT	9.59	days
	VFA [C]	9.65	g/L	Liq Flow rate (after sampling)	1.482	L/day
Global	degradation efficiency	56%				
C2	**Working volume**	**10**	**L**			
	Reactor productivity	5.20E-02	g/L/h	[JF Cornet - October 2007]		
	Theoretical VFA load			Theoretical HRT		
	Simulation VFA Load	1.244		Simulation HRT	0.910	days
	VFA removal efficiency	100%		Liq Flow rate (after sampling)	10.99	L/day
	Biomass [C]	1.08	g/L			
C3	Working Volume	1	L			
	Theoretical N load	1.6	g/L/d (maximum?)	Theoretical HRT		
	Simulation N Load	0.446	g/L/d	Simulation HRT	0.091	days
	nitrification efficiency	98%		Liq Flow rate (after sampling)	10.49	L/day
	Biomass [C] -----		g/L			
C4	Working Volume	8	L			
	Reactor productivity	1.53E-02	g/L/h	[JF Cornet - October 2007]		
	Co2 removal efficiency	LIMITING		Theoretical HRT		
	HNO3 remova	64%		Simulation HRT	0.763	days
	Biomass [C]	0.24	g/L	Liq Flow rate (after sampling)	9.99	L/day

3. Scenario 1

Scenario 1 started from the following assumptions for compartment hardware:

- Compartment I includes a steam-sterilizable filtration unit. All upstream parts are not steam-sterilizable.
- Based on the evaluation performed in TN80.21-23, compartments II to IVa combine autoclavable photoreactors with steam sterilizable side equipment. The requirements for sterile operation, minimal material leaching, optimal process control and datalogging,… imply the need for a high-tech pilot-scale installation. It was therefore decided to contact suppliers of commercial bioreactors with clear experience in the pharmaceutical industry.

3.1. First round of contacts

3.1.1. Waste preparation unit and Compartment I

EPAS was subcontracted in the BELISSIMA contract to engineer and construct the first compartment. They work with a construction company that was also involved in the MELiSSA-contract Engineering of the Waste Compartment. Their quotation for the waste preparation unit amounted to 47 kEUR. The quotation for compartment I including an influent buffer tank, a bioreactor coupled to a filtration unit, a filtrate tank and a gas loop, amounts to 232 kEUR (Ra 0.8) or 341 kEUR (Ra 0.5). The total cost of 232 kEUR consists of 128 kEUR mechanical parts, sensors and actuators, 13 kEUR electronic materials, and 91 kEUR manpower for supervision, construction, programming, etc.

3.1.2. Compartment II to CIVa

For the compartments CII till CIVa, VITO contacted four suppliers in a first round: Sartorius, Applikon, New Brunswick Scientific and Bioengineering. They were provided with requirement documents for the different reactors. They were, in a first phase, asked to provide a detailed offer for CII, including an influent and effluent vessel.

Several meetings with the different suppliers were required to get a good understanding of their possibilities in constructing this type of pilot-scale installations. Table 6 provides an overview of this first round of contacts. For this first selection, attention was paid to the course of the meetings, the provided input for remaining open questions in design, the ability to meet dead-lines and of course the quotations, completeness of the proposals, delivery times, etc.

From the meetings with New Brunswick Scientific it quickly became clear that they would not be able to provide a good solution for the BELISSIMA project. As a result, they were not retained for further contacts.

Direct meetings with Bioengineering were difficult, due to the distance. Their representative in The Netherlands acted mainly as a messenger to the headquarters. Discussions were far less constructive than with Sartorius and Applikon. The first proposal showed important incompletenesses. The cost was higher compared to the other two proposals.

Table 6: General information suppliers and findings during first round of contacts

	Sartorius	**Applikon**	**Bioengineering**	**New Brunswick Scientific**
Head office	Germany	The Netherlands	Switzerland	America
Nearest representative	Sartorius Belgium	Applitek (Belgium)	Distrilab (The Netherlands)	Belgium
Contact persons	Jozeph Peeters (B, has left) Davy De Wilde (B) Wilhelm Bernd-Ulrich (D) Wolf-Dietrich Linke (D)	Lievin Martens (B) Edwin Krowinkel (NL) Michel Kensler (NL) Ulrich Hünnemeyer (NL)	Ferrie Soet (NL) Judith Laws (CH)	Luc Tourwé (B)
In general	✓ clearly interested in this type of projects ✓ strong technical knowledge ✓ flexible solutions	✓ clearly interested in this type of projects ✓ strong technical knowledge ✓ flexible solutions	✓ appear less interested in this type of 'smaller' projects ✓ more difficult direct contact with headquarters	✓ are not able to provide a 'tailor-made' solution for the BELISSIMA loop ✓ appear less interested in this type of 'smaller' projects
Course of the meetings	✓ four meetings with intensive discussion of the requirements, their possibilities, new ideas,… ✓ able to provide good solutions for open questions	✓ four meetings with intensive discussion of the requirements, their possibilities, new ideas,… ✓ able to provide good solutions for open questions	✓ Distrilab acted only as 'messenger' ✓ only once meeting with representative of Bioengineering ✓ minor technical input on open questions	✓ two brief meetings to check the possibilities of providing solutions for the BELISSIMA project
First proposal CII	✓ proposal close to requirements, some issues however missing (gas analyser, steam sterilisable connection valves,…) ✓ standard 10 liter fermentor (19 cm diamers) as starting point	✓ proposal close to requirements, some issues however missing (illumination, gas analyser, training,…) ✓ can provide a tailor-made 16 cm diameter reactor	✓ proposal differs from requirements ✓ use of 20 cm standard reactor	✓ Only price for standard reactor

	Sartorius	Applikon	Bioengineering	New Brunswick Scientific
Tentative quotation	~ 350 000 EUR	~300 000 EUR	~360 000 EUR	-
Remarks	✓ detailed proposal, however without process flow diagram	✓ without illumination ✓ without training ✓ brief proposal, although including component list and process flow diagram	✓ incomplete proposal ✓ misunderstanding of the requirements in some parts ✓ a 16cm tailor made reactor would cost additionally 33000 euro	
Dead-lines	✓ have exceeded all dead-lines ✓ it appears difficult to get information from German headquarters in time. However, always quick response from Belgian representative.	✓ have exceeded deadlines slightly	✓ proposal was delivered in time	-
Delivery time	5 months	4 months	10 months	-
Other issues	✓ SS in vessels: Ra <0,8µm	✓ SS in vessels: Ra <0,4 µm		-

3.2. Second round of contacts for compartments II and III

Both Sartorius and Applikon were asked to provide a detailed and more complete quotation for compartments CII and CIII, including process flow diagrams, separate costs for engineering phases,…

Table 7 provides some important issues for comparison of the remaining suppliers Sartorius and Applikon, concerning compartment CII. An illumination device is not included in the proposals. Cleaning in place (CIP) and steaming in place connections are foreseen, but the steam generator and CIP unit or an autoclave for sterilization of the glass part are not included.

Table 8 provides the findings concerning the preliminary proposals for compartment CIII. CIP and steaming in place connections are foreseen, but the steam generator and CIP unit are not included. Although the prices are lower, the concept proposed by Applikon with a reactor consisting of 3 parts, seems less interesting.

Prices given in the budget proposals ranged from €260 000 to €400 000 per compartment. Important cost factors in these proposals are the chosen high-level materials and the option of cleaning and steaming in place.

Table 7: Comparison between proposals for CII of Sartorius and Applikon

	Sartorius	**Applikon**
Quotation CII	**~ 330 000 euro**	**~ 275 000 euro**
Including	✓ BIOSTAT B-DCU (€ 89 000) ✓ 2 pressure vessels (€ 202 000) ✓ 2 balances (€ 21 000) ✓ transport (€ 2 500) ✓ training (€ 4 500) ✓ additional engineering, CO_2 probe, … as given in revision of proposal (€ 11 400)	✓ bioreactor ✓ 2 * 40 l vessels ✓ 2 * iControl system
Impression	✓ detailed offer, however ✓ no process flow diagram, although mentioned that they would provide ✓ no clear quotation, revisions are given as annex of previous versions	✓ simple basic offer ✓ details given in Excel component list ✓ clear and detailed process flow diagram
Engineering costs	**~ 12 000 euro**	**37 647 euro**
Delivery time	~ 4 months after placing the order and clarification of all technical details	10 to 14 weeks, after clarification of all technical details
Missing?	✓ special sterilisable connection valves? ✓ conductivity probe?	✓ thermostatic bath for reactor?
Questions	✓ exact engineering cost? ✓ what is exactly included in engineering?	✓ high costs engineering ? What is exactly included? ✓ temperature control of fermentor?
Important issues	✓ Ra < 0,8 µm ✓ illumination device would cost ~15 000 euro ✓ Sartorius uses balances for level control in in- and effluent vessel	✓ Ra < 0,4 µm

Table 8: Comparison between proposals for CIII of Sartorius and Applikon

	Sartorius	**Applikon**
Quotation CII	**414 000 euro**	**258 918 euro** (option 1: reactor split into three parts) **263 059 euro** (option 2: one reactor in 3 parts, partly glass, partly SS) **334 765 euro** (option 3: complete SS reactor in 3 parts)
Including	✓ BIOSTAT C-DCU - microcarrier (€ 172 000) ✓ 2 pressure vessels (€ 220 500) ✓ balance (€ 10 500) ✓ transport (€ 4 500) ✓ training (€ 6 500)	✓ packed bed reactor ✓ 2 * 60(70) l vessels ✓ 2 * iControl system
Impression	✓ detailed offer, however ✓ no process flow diagram, although mentioned that they would provide ✓ difficult to evaluate on its completeness	✓ simple basic offer ✓ details given in Excel component list ✓ preliminary process flow diagram, only of reactor ✓ difficult to evaluate on its completeness
Engineering costs	**12 000 euro (CIII)**	**37 647 euro (option 1)** **38 824 euro (option 2)** **47 059 euro (option 3)**
Questions	✓ in- and effluent vessel more expensive (+20 000 euro), compared to those for CII?	
Important issues	✓ 40l vessels: SS with Ra of 0,8 µm ✓ culture vessel: SS with Ra <0,5 µm	✓ 70l vessels! Ra <0,4 µm ✓ bioreactor in SS Ra <0,8 µm ✓ SS piping inside Ra <0,8 µm

4. Scenario 2

As the quotations for scenario 1 exceeded the project budget for hardware drastically, ESA and VITO agreed to investigate an alternative approach starting from basic fermentor units and providing them with side-equipment in a flexible way. This implied

- use of autoclavable equipment instead of steaming in place for sterilization. Autoclavable bioreactors are still used because this allows 100% illumination of the photoreactors.
- set-back from strict use of pharmaceutical grade materials.

Flexibility is needed throughout the BELISSIMA study. Due to its complexity, it is anticipated that the test program and results may on the long term reveal the need for e.g. extra sensors or adaptations in the set-up. The flexibility in the revised design approach is situated in the following aspects:

- Compartment working volume: the compartment vessels will have the flexibility to operate at two different working volumes; one for stand alone operation and one for coupled set-up. In the former case, plenty of effluent is available for microcompound analyses and the test program is in principle limited to minerals for which the required sampling volumes are low (see TN80.15). Large throughputs are not needed at that stage and manpower for manipulation of buffer tanks and preparation of media can be rationalized by operating at a lower volume. In the latter case, larger sample volumes may be required and operation at a high working volume may be needed.
- Access ports: additional access ports will be provided on all reactor vessels to allow for the implementation of additional sensors, sampling, etc when this is required.
- Tubing: the use of tubing instead of stainless steel piping allows to upgrade the system any time at a reasonable cost. Tubing can easily be replaced on a regular basis to prevent biofilm formation.

In light of the above, Applikon and Sartorius were asked to provide new quotations for CII, starting from standard equipment with standard control. For compartment I, quotations were asked from EPAS and alternatively from the company Saillart for the reactor vessels.

4.1. Modifications design

Per compartment, the following design changes were proposed.

Compartment I:

- Influent tank and bioreactor in glass instead of stainless steel
- Tubing
- No redundancy of membrane module.

The membrane unit will still be steam sterilized.

Compartment II:

- Working volume variable between 5 and 10 l. Stand-alone operation at 5 l working volume. Operation at 10 l when needed with connected compartments or for operation in closed loop.
- Influent and effluent vessel in glass
- Tubing.

For compartments III and IVa the changes are similar and are detailed in 4.2.

4.2. Implications on requirements BELISSIMA study

Table 9 to Table 13 summarize the BELISSIMA requirements listed in TN80.16 and evaluate the impact of the proposed design changes on the requirements. Impact on laboratory requirements is not discussed since this is not affected.

4.2.1.Compartment I

Table 9: Impact of design changes on BELISSIMA requirements for compartment I. The numbers between brackets refer to the relevant paragraph in TN80.16.

Requirement	Impact of design change and proposed countermeasure
General requirements (2.1.1)	No impact
Process requirements (2.1.2)	No impact
System requirements (2.1.3)	
- feeding	Due to the presence of only one filtration unit, the permeate production will be temporarily interrupted during steaming in place. The total permeate production per day will have to be in line with the required hydraulic retention time. The total membrane surface area installed will have to be sufficiently large to allow for temporary increases in flux to compensate for the downtime. Feeding will be semi-continuous as for the Pilot Plant CI.
- automation	No impact
- sterility	No requirement that reactor is sterile. Sterility filtration loop maintained through steam sterilization.
- gas closure	No impact
- pressure	No impact
- pH correction	No impact
- gas flow	No impact
- reactor size, stirring and heating	No impact
- measurements of flow, temperature, pH, pressure	No impact
- buffering vessels	Glass vessels will have to be covered to minimize illumination and potential algal growth.
- feed preparation unit	No impact
Control (2.1.4)	
- pH, temperature, pressure	No impact
- level	During membrane cleaning, a temporary level increase may be implemented or the feed flow may have to be interrupted to avoid a level increase.
- approach	No impact
Maintenance (2.1.4)	Sterilization of sampling ports is only an issue at the permeate side of the membrane filtration unit.

4.2.2.Compartment II

Table 10: Impact of design changes on BELISSIMA requirements for compartment II. The numbers between brackets refer to the relevant paragraph in TN80.16.

Requirement	Impact of design change and proposed countermeasure
General requirements (2.2.1)	No impact
Process requirements (2.2.2)	No impact
Sampling requirements (2.2.2)	No impact
System requirements (2.2.3)	
- feeding	No impact
- automation	No impact
- cleaning	No impact
- sterility	This will be achieved through autoclaving rather than by steam sterilization. All parts will be sterilizable. Chances of contamination will be higher through an increase in manipulations. Sterile connections are therefore foreseen. The personnel will be trained in sterile/axenic work.
- gas closure	No impact
- pressure	No impact
- pH correction	No impact
- gas flow	No impact
- reactor active volume	The working volume will be flexible. It will be 5 l when the compartment operates separately. When the different compartments are connected and large sample volumes are needed, it will be increased to 10 l. All side-equipment will be able to operate in both ranges.
- reactor shape	No impact
- reactor diameter	No impact
- illumination	No impact. The illumination system will be designed such that the required light intensity is achieved both at 5 and 10 l working volume.
- stirring and cooling	No impact. Stirring blades will be positioned such that mixing for both working volumes is optimal.
- measurements of flow, temperature, pH, pressure, level, light intensity	No impact
- biomass concentration measurement	Will not be available. Since CII is a completely mixed reactor, the effluent should ideally have the same biomass concentration as the reactor content. This will be verified through parallel sampling of reactor content and effluent. The frequency of off-line biomass measurements will be increased. An additional sampling port will be available to allow for implementation of the sensor at a later stage, when this is deemed necessary.
- conductivity measurement	Will not be available. Conductivity does not tend to fluctuate strongly. Furthermore the conductivity of the reactor content and effluent should be similar due to the completely stirred character of the compartment. With respect to the microcompound studies, element analyses will in any case be more important than the overall conductivity measurement. An additional sampling port will be available to allow for implementation of the sensor at a later stage, when this is deemed necessary.
- redox measurement	This measurement is not expected to be crucial. Moreover, redox measurements tend to be difficult to interpret. In any case, an additional sampling port will be available to allow implementation of the sensor at a later stage, when this is deemed necessary.
- off-gas measurements	No impact
Material requirements (2.2.3.1)	Resistance to steam sterilization is no longer required. All the other requirements are attained when using glass vessels.

	By the replacement of stainless steel piping with flexible plastic tubing, leaching of organic compounds may be an issue. This will be reduced by the use of high grade tubing, which is also accepted in pharmaceutical industry. If leaching turns out to be a problem, the design will be adapted towards stainless steel piping. The replacement of stainless steel vessels with glass ones is advantageous since it reduces the risk of metal leaching and avoids the need for specific coatings which can release organic contaminants.
Interfaces (2.2.3.2) - buffering vessels - harvesting system	 The buffer vessels will have a volume of 50 l, corresponding to the needs for the maximal CII volume of 10 l. The buffer vessels will be glass ones. They will have to be protected from light. Due to more complex handling, the risk of contamination will be higher than for stainless steel vessels which allow for SIP. No impact
Control (2.2.4)	No impact. Both the standard control provided by the suppliers and PLC control allow proper control and operation of the compartment.
Maintenance (2.2.4)	Aspects such as accessibility, sterilization and axenic disconnection are not affected.

4.2.3.Compartment III

Table 11: Impact of design changes on BELISSIMA requirements for compartment III. The numbers between brackets refer to the relevant paragraph in TN80.16.

Requirement	Impact of design change and proposed countermeasure
General requirements (2.3.1)	No impact
Process requirements (2.3.2)	No impact
Sampling requirements (2.3.2)	No impact
System requirements (2.3.3) - feeding - recirculation - backwash - sterility - gas closure - pressure - pH correction - gas flow - reactor size - reactor shape - fixed bed - stirring and T control - measurements of flow, temperature, pH, oxygen, pressure, level - off-gas measurements	 No impact No impact No impact This will be achieved through autoclaving rather than by steam sterilization. All parts will be sterilizable. Chances of contamination will be higher through an increase in manipulations. Sterile connections are therefore foreseen. The personnel will be trained in sterile/axenic work. No impact No impact No impact No impact As for compartment II, the working volume for CIII could be chosen differently for stand alone or coupled operation. However, due to the risk of contamination related to the required addition of extra carrier material and the slow growth of the nitrifying organisms, preference is given to using a fixed working volume. Sizing will be based on the maximal CII working volume of 10 l and is then approximately 1 l. No impact No impact No impact No impact No impact

Material requirements (2.3.3.1)	Resistance to steam sterilization is no longer required. All the other requirements are attained when using glass vessels. By the replacement of stainless steel piping with flexible plastic tubing, leaching of organic compounds may become an issue. This will be reduced by the use of high grade tubing, which is also used in the pharmaceutical industry. If leaching turns out to be a problem, the design will be adapted towards stainless steel piping.
Interfaces (2.3.3.2) - buffering vessels - harvesting system	 No impact on size. Since the buffer vessels will be glass ones, they will have to be protected from light. No impact
Control (2.3.4)	No impact. Both the standard control provided by the suppliers and PLC control allow proper control and operation of the compartment.
Maintenance (2.3.4)	Aspects such as accessibility, sterilization and axenic disconnection are not affected.

4.2.4.Compartment IVa

Table 12: Impact of design changes on BELISSIMA requirements for compartment IVa. The numbers between brackets refer to the relevant paragraph in TN80.16.

Requirement	Impact of design change and proposed countermeasure
General requirements (2.4.1)	No impact
Process requirements (2.4.2)	No impact
Sampling requirements (2.4.2)	No impact
System requirements (2.4.3) - feeding - automation - sterility - gas closure - pressure - pH correction - gas flow - reactor size - reactor shape - reactor diameter - illumination - stirring and cooling - measurements of flow, temperature, pH, pressure, oxygen, light intensity - biomass concentration measurement	 No impact No impact This will be achieved through autoclaving rather than by steam sterilization. All parts will be sterilizable. Chances of contamination will be higher through an increase in manipulations. Sterile connections are therefore foreseen. The personnel will be trained in sterile/axenic work. No impact No impact No impact No impact The working volume will be different when the compartment operates separately or in coupled set-up. It will be maximal when the different compartments are connected and large sample volumes are needed (current estimate 11 l). It will be halved during stand alone operation to limit the efforts for synthetic medium preparation. All side-equipment will be able to operate in both ranges. No impact No impact No impact. The illumination system will be designed such that the required light intensity is achieved for both working volumes. No impact. Stirring blades will be positioned such that mixing is optimal for both working volumes. No impact Will not be available. Since CIVa is a completely mixed reactor, the effluent should ideally have the same biomass concentration as the reactor content. This will be verified through parallel sampling of reactor content and effluent. The frequency of off-line biomass measurements

	will be increased. An additional sampling port will be available to allow for implementation of the sensor at a later stage, when this is deemed necessary.
- off-gas measurements	No impact
Material requirements (2.4.3.1)	Resistance to steam sterilization is no longer required. All the other requirements are attained when using glass vessels. By the replacement of stainless steel piping with flexible plastic tubing, leaching of organic compounds may become an issue. This will be reduced by the use of high grade tubing, which is also used in the pharmaceutical industry. If leaching turns out to be a problem, the design will be adapted towards stainless steel piping. The replacement of stainless steel vessels with glass ones is advantageous since it reduces the risk of metal leaching and avoids the need for specific coatings which can release organic contaminants.
Interfaces (2.4.3.2) - buffering vessels	No impact on size. As the buffer vessels will be glass ones, they will have to be protected from light.
- harvesting system	No impact
Control (2.4.4)	No impact. Both the standard control provided by the suppliers and PLC control allow proper control and operation of the compartment.
Maintenance (2.4.4)	Aspects such as accessibility, sterilization and axenic disconnection are not affected.

4.2.5.General requirements

Table 13: Impact of design changes on general BELISSIMA requirements. The numbers between brackets refer to the relevant paragraph in TN80.16.

Requirement	Impact of design change and proposed countermeasure
Overall requirements (2.5)	
power supply, signals	No impact
steam line for sterilization	No change. Still required for sterilization of the membrane filtration unit permeate line of CI. For sterilization of the autoclavable vessels, a large autoclave is needed.
automation and operation control	No impact
valves and connections	No impact. Sufficient ports will be foreseen to allow for future implementation of additional probes.
harmonized hardware	No impact
safety	Due to the need of axenic operation, the use of autoclavable equipment will require more manipulations and the risk of contamination increases.
Compartment operation (2.6)	
optimal operation	No impact
operation at constant working volume	Although the working volume may change between stand alone operation and coupled set-up, it will always remain constant during a test period.
Microcompound study	
critical issues (3.1)	No impact on pH correction and adequate measurement dosed amounts of acids and bases
feed to compartment I (3.2)	No change in feed composition or processing
definition of strains (3.3)	No impact
sampling conditions (3.4)	No impact
protocols (3.5)	No impact

4.3. Implications in terms of costs

Although a reduction in hardware costs can be anticipated for the revised design scenario, it is important to include all costs associated with construction and operation of the BELISSIMA loop over a sufficiently long period of time. To this end, the costs of scenarios 1 and 2 were compared. In summary:

- scenario 1: autoclavable bioreactor + steam sterilizable associated equipment,
- scenario 2: autoclavable bioreactor + autoclavable associated equipment.

In a first paragraph, some general aspects of changing from scenario 1 to 2 are evaluated in terms of costs, which are common for various compartments. In the subsequent paragraphs, specific cost aspects for the individual compartments are considered.

4.3.1.Impact of general issues on costs

- Cover for (glass) buffer tanks to protect from light: negligible cost

- Replacement of stainless steel piping with flexible plastic tubing: the cost aspects are included in the overall cost comparison of both scenarios (see below). The risk of leaching metals from the stainless steel is now replaced by the risk of leaching organic microcompounds from tubing. This will be minimized by the use of specific pharmaceutical grade tubing. Furthermore, if the leaching turns out to be problematic for the microcompound analysis, stainless steel piping can still be implemented.

- Off-line versus on-line gas analysis
  On-line analysis concerns the gases CO_2 and CH_4 for CI, CO_2 for CII, O_2 and CO_2 for CIII and CIVa. In principle, CO_2 will be measured by IR analysis and O_2 by paramagnetism. Gas analysis requires pretreatment to remove the humidity.
  For CH_4 determination, both FID and IR are valuable options. Table 14 shows that FID analysis offers the highest accuracy. However it is less preferable in the BELISSIMA context for the following reason. The headspace in the compartment is limited and gas losses should be minimized. Because the flow rates needed for analysis are high, operation in a closed gas loop is preferable. Due to the addition of carrier gases and the destructive nature of the FID analysis, the gas composition in compartment II would be affected in a closed loop.

  IR analysis offers the advantage that it allows analysis of both CH_4 and CO_2 at a sufficiently large accuracy. Furthermore, it could be used for CO_2 analysis in all 4 compartments. When a paramagnetic O_2 measurement is included, 1 system could even be used for all on-line analyses. The disadvantage is that applying one system over the broad concentration range expected between the different compartments will reduce its accuracy outside the optimal range. It may therefore be advantageous to use different lower cost systems with a sufficient accuracy in different concentration ranges.

Table 14: Evaluation of FID and IR analysis in the BELISSIMA loop

	FID analysis	**IR analysis**
destructive analysis	yes	No
compounds	CH_4	CH_4 and CO_2
required flow	1 to several l/min	1 to several l/min
sensitivity	linear in a broad range high sensitivity	specific cuvette needed for different measurement ranges lower sensitivity
gas phase losses	substantial	limited
gas phase composition	affected because H_2 or He/H_2 are used as carrier gas	not changed
application	compartment I	compartment I to IVa
cost	12.500 EUR	6.000 to 12.500 EUR

Sharing one analyzer for different compartments requires a valve to switch between gas streams. In between two samplings, the valve should be flushed with He. When it switches to one of the compartments, a limited amount of He may thus enter the gas phase but this is not problematic since He is also used to keep the BELISSIMA compartments at a slight overpressure.

When the calculated costs are considered for an operational period of several years, the on-line analyses are competitive and offer the additional advantage of a higher measurement frequency (see Table 15). Preference is given to an IR analyzer (as also suggested in TN71.5). Presumably it is better to provide two low cost analyzers instead of one high cost analyzer, because this allows a better accuracy in various concentration ranges (40-80% CO_2 in CI as opposed to several 100 C-ppm in CIVa). This is expected to give a similar cost.

Table 15: Cost comparison of on-line and off-line gas analysis

CI	**on-line gas analysis**	**off-line gas analysis**
type	$CH_4 + CO_2$: IR	CH_4: FID, CO_2: IR
frequency	CH_4 + CO_2: assuming that equipment is shared for compartments I to IVa, once per hour	3/week (TN80.16)
cost	IR: 3.150 EUR (1/4 of total cost which is shared over 4 compartments)	120 EUR/week ~5000 EUR/yr
CII		
type	CO_2: IR	CO_2: IR
frequency	CO_2: assuming that equipment is shared for compartments I to IVa, once per hour	1/day (TN80.16)
cost	IR: 3.150 EUR (1/4 of total cost which is shared over 4 compartments)	75 EUR/week ~3750 EUR/yr
CIII		
type	CO_2: IR O_2: paramagnetic	CO_2: IR O_2: paramagnetic
frequency	CO_2 + O_2: assuming that equipment is shared for compartments I to IVa, once per hour	1/day (TN80.16)
cost	Since O_2 measurement can be incorporated in the IR apparatus, the costs are identical as for CO_2 measurement alone: 3.150 EUR (1/4 of total cost which is shared over 4 compartments)	75 EUR/week ~3750 EUR/yr

CIVa		
type	CO_2: IR O_2: paramagnetic	CO_2: IR O_2: paramagnetic
frequency	CO_2 + O_2: assuming that equipment is shared for compartments I to IVa, once per hour	1/week (TN80.16)
cost	Since O_2 measurement can be incorporated in the IR apparatus, the costs are identical as for CO_2 measurement alone: 3.150 EUR (1/4 of total cost which is shared over 4 compartments)	15 EUR/week ~750 EUR/yr
total cost	12.500 + 2.000 (Peltier cooler) + 2.500 (streamswitcher) = 17.000 EUR	~9.500 EUR/yr

4.3.2.Compartment I

- Accommodate for cleaning single membrane filtration unit
  The design of the first compartment in the MELiSSA Pilot Plant includes two parallel membrane modules which each have a surface area of 0.021 m². Through a parallel set-up, continuous production of permeate is ensured even during cleaning or sterilization. At a hydraulic retention time of 10 d and a bioreactor volume of 100 l, the flowthrough is 10 l/d which corresponds to a flux of around 20 l/m².h.

  In BELISSIMA, the first compartment will have a volume of around 20 l. At a HRT of 10 d, this corresponds to a flow of 2 l/d. Assuming the same membrane module at a surface area of 0.021 m², fluxes would be extremely low. When operation at 15 l/m².h were considered the maximum under the same conditions, the membrane surface area could be reduced to 0.008 m². The use of a ceramic membrane allows for steam sterilization of the membrane filtration unit.

- Costs for revised design requirements
  EPAS provided a revised quotation for the first compartment, now using a glass reactor instead of a stainless steel one and using tubing instead of stainless steel piping. The filtration unit contains a single membrane module and remains steam sterilizable. VITO made a cost estimate for the same P&ID. Table 16 shows the major cost categories. On the one hand, the choice of components is different and leads to higher hardware costs for the VITO cost estimate. On the other hand, even though VITO reduced the manpower requirements to the absolute minimum, its manpower costs are not competitive with EPAS.

Table 16: Cost estimates for Compartment I

	quotation EPAS	estimate VITO
Mechanical/sensors/actuators	**56 kEUR**	**115 kEUR**
Blender	2.8 kEUR	6 kEUR
Flow sensors	3.8 kEUR	1.5 kEUR
Level switches and sensors	1 kEUR	2 kEUR
Membrane	3 kEUR	1 kEUR
pH control	1.7 kEUR	11.6 kEUR (including 2 balances)
Pressure regulators	2.6 kEUR	7.5 kEUR
Pumps	6.5 kEUR	8 kEUR
Tanks and vessels	14.5 kEUR	28 kEUR
Temperature sensors	1.3 kEUR	2 kEUR
Valves	14.8 kEUR	7 kEUR
Tubings, connectors, frames, etc.	4 kEUR	39 kEUR
Materials electronics	**13.4 kEUR**	**47 kEUR**
PLC	5.3 kEUR	20 kEUR (including PC, subcontracted)
Electronics	8.1 kEUR	27 kEUR (including electrical cabinet, partly subcontracted)
Manpower	**34 kEUR**	**130 kEUR**
Supervision	6.7 kEUR	14 kEUR
Component analysis, ordering	/	38 kEUR
Construction/integration	10.7 kEUR	58 kEUR
Wiring	3.2 kEUR	included under electronics
Programming	6.4 kEUR	6.4 kEUR (when done by EPAS)
Trouble shooting	4 kEUR	9 kEUR
Testing	3 kEUR	5 kEUR
Total	**104 kEUR**	**292 kEUR**

The cost of the waste preparation unit is not included and amounts at least to another 50 kEUR. The change in material does not impact the resources needed for compartment operation since sterility requirements remain the same.

4.3.3.Compartment II

- Steam-sterilizable versus autoclavable hardware: The switch from steam sterilizable to autoclavable hardware has an impact on the risk of contamination as well as on the supply strategy and the assembly and operation of the compartment. Both the contamination risks and supply strategy are discussed before the overall cost comparison is performed.

Contamination risk and operational aspects

Operation of compartment II will consist of the following actions:

- Scenario 1: autoclavable bioreactor + steam sterilizable associated hardware
    1. autoclaving the bioreactor (possibly with synthetic medium for start up)

2. steam sterilizing the influent and effluent tanks and piping to and from the bioreactor
3. connecting the steam sterilized side-hardware to the autoclaved bioreactor through sterile couplings
4. connecting the gas phase supplies and the autoclaved acid and base dosing units
5. inoculation of the bioreactor
6. autoclaving of synthetic medium
7. sterile transfer of autoclaved synthetic medium to the influent tank
8. start up of continuous operation
9. autoclaving of batches of synthetic medium
10. sterile transfer of autoclaved synthetic medium to the influent tank (possibly after intermediate steam sterilization, although this would require emptying the influent tank and interruption of the continuous fermentation process)
11. when shift to real permeate from compartment I, transfer of filter-sterilized compartment I permeate (possibly after intermediate steam sterilization)
12. steam sterilization of effluent tank when deemed necessary
13. autoclaving bioreactor and new start up when major contamination or problem occurs
14. sampling from bioreactor and effluent tank.

- Scenario 2: autoclavable bioreactor + autoclavable associated hardware
    1. autoclaving the bioreactor (possibly with synthetic medium)
    2. autoclaving the influent and effluent tanks and tubing to and from the bioreactor
    3. connecting the autoclaved side-hardware to the autoclaved bioreactor through sterile couplings
    4. connecting the gas phase supplies and the autoclaved acid and base dosing units
    5. inoculation of the bioreactor
    6. autoclaving of synthetic medium
    7. sterile transfer of autoclaved synthetic medium to the influent tank
    8. start up of continuous operation
    9. autoclaving of batches of synthetic medium
    10. connection of autoclaved influent tank to bioreactor through sterile coupling or sterile transfer of autoclaved synthetic medium to the influent tank (possibly after autoclaving the influent tank and interruption of the continuous process)
    11. when shift to real permeate from compartment I, transfer of filter-sterilized compartment I permeate (possibly after intermediate autoclaving of the influent tank and interruption of the continuous process)
    12. autoclaving effluent tank when deemed necessary
    13. autoclaving bioreactor and new start up when major contamination or problem occurs
    14. sampling from bioreactor and effluent tank.

The frequency and risks of the manipulations are indicated in Table 17. It shows that in principle the number of manipulations is similar for both scenarios, however the contamination risk differs. The main differences between both scenarios lie in actions

- 3 (coupling of sterilized hardware)
- 7 and 10 (transfer of autoclaved medium to sterilized hardware)
- 14 (sampling from bioreactor and effluent tank).

The contamination risk due to sampling is also related to the sampling frequency, which depends on the presence or absence of on-line sensors or analyzers. In most cases, the cost of on-line sensors is competitive with off-line analysis when considered over a sufficiently long period of time (see Table 15, Table 21, Table 22, and Table 25). Therefore, the sampling frequency can be considered equal to scenario 1.

Table 17: Frequency and contamination risk for 2 sterilization scenarios. Scenario 1: autoclavable bioreactor + steam sterilizable associated hardware, scenario 2: autoclavable hardware. Differences in risks are indicated in bold. For scenario 2, the presence of on-line sensors and analysers has been assumed.

action	**scenario 1**	**scenario 2**
1. sterilizing bioreactor	autoclaving at start-up empty reactor: very low risk	autoclaving at start-up empty reactor: very low risk
2. sterilizing influent and effluent tank and piping to and from bioreactor	steam sterilization at start-up empty tanks: very low risk	autoclaving at start-up empty tanks: very low risk
3. connecting sterilized bioreactor and tanks	at start-up steam sterilizable sterile couplings: very low risk	at start-up sterile couplings: **low to intermediate** risk
4. connecting gas supplies and acid/base dosing units	at start-up very low risk	at start-up very low risk
5. inoculation of bioreactor	at start-up very low risk	at start-up very low risk
6-9. sterilizing synthetic medium	autoclaving 3-4/week very low risk	autoclaving 3-4/week very low risk
7-10. sterile transfer of medium to influent tank	3-4/week low risk	3-4/week **low to intermediate risk**
8. start-up of continuous operation	at start-up very low risk	at start-up very low risk
11. transfer of real CI permeate	5/week low to intermediate risk	5/week low to intermediate risk
12. sterilizing effluent tank	frequency unknown very low risk	frequency higher very low risk
13. contamination bioreactor	1/yr low risk	3/yr **intermediate** risk but with steam sterilizable couplings: 1/yr, low risk
14a. sampling from effluent tank	3/week through steam sterilizable port very low risk	3/week through sampling port **low to intermediate** risk
14b. sampling of bioreactor	weekly through steam sterilizable port very low risk	weekly through sampling port **low to intermediate** risk

The higher contamination risk associated (in scenario 2) with the coupling of the sterilized influent and effluent tanks to the autoclaved bioreactors, the transfer of autoclaved medium and sampling can be reduced by

- Using steam sterilizable connections or a mobile laminar air flow to proceed under sterile air: the first option is the most flexible one and gives the highest risk reduction. A mobile laminar air flow cabinet cannot always be positioned in between influent and bioreactor or bioreactor and effluent tanks. Proper positioning for sterile sampling is even more problematic.
- Training of the personnel in axenic and sterile work.

Based on the above, the risk of contamination and the need to restart the loop will be higher in scenario 2. In such case, the time loss can quickly amount to 1 month. This corresponds to an additional cost of ~10 kEUR per event or an extra yearly cost of 20 kEUR for scenario 2 as compared to scenario 1. In case steam sterilizable couplings and sampling ports can be included, the contamination risk can be reduced. The extra hardware costs for 2 sampling ports (1 on bioreactor and 1 on effluent tank), 1 coupling to the influent and one to the effluent tank and 1 coupling at influent tank for transfer of medium) are estimated to be 25 kEUR. However, none of the contacted suppliers wishes to offer a combination of steam sterilizable couplings and autoclavable hardware because of too high safety risks.

Supply strategy and assembly

In scenario 1, a near-complete unit including influent and effluent tanks and piping is delivered by 1 supplier and VITO does not have to do any engineering work. The corresponding supplier quotations have been compared in Table 7.

In scenario 2, the bioreactor is delivered by one supplier, whereas the influent and effluent tanks are provided by another one. VITO then would perform the final assembly. For the bioreactor system, quotations were asked both from Sartorius and Applikon. Table 18 shows that Sartorius is clearly the cheapest supplier, probably because its standard UniVessel has the required 19 cm internal diameter, whereas Applikon has to provide this tailor-made. Moreover, the offer from Sartorius is the most detailed one providing prices for all individual items as requested.

Table 18: Comparison of Sartorius and Applikon quotations for revised requirements

	Sartorius	**Applikon**
Quotation CII	**~ 56 500 euro**	**~ 78 500 euro**
Including	✓ BIOSTAT B-DCU for 4 supply towers but including 1 ✓ UniVessel 10 l (€ 5 353) ✓ Pump, temperature and electrical module (€ 11 000) ✓ Gassing modules He-CO2 (€ 6 700) ✓ Air exhaust (€ 1 000) ✓ Samplers + sterile connectors (€ 2 000) ✓ pH, level, pressure measurement (€ 2 500)	✓ 10 l autoclavable bioreactor ✓ Gassing module ✓ Temperature, level, pH measurement

	Sartorius	Applikon
	✓ Local control system DCU-Tower (€ 17 000) ✓ Project specific software configuration (€ 1 500) ✓ 3 balances (€ 3 200) ✓ Transport, installation, training (€ 1 530)	✓ 1 iControl system ✓ 2 balances
Impression	✓ detailed offer ✓ standard 19 cm diameter reactor vessel	✓ basic offer without cost details ✓ no update Excel component list ✓ no update process flow diagram
Delivery time	14-16 weeks	10 to 14 weeks
Missing	✓ Influent and effluent tank with sensors/transmitters ✓ Illumination device	✓ Influent and effluent tank with sensors/transmitters ✓ Illumination device ✓ Stand alone pump for feeding and effluent ✓ Backpressure control valves
Important issues	✓ Ra < 0,8 µm ✓ Balances for level control ✓ Magnetic coupling stirrer-vessel	✓ Ra < 0,8 µm ✓ Pumps at preset flow ✓ Magnetic coupling stirrer-vessel

For the overall cost comparison of scenarios 1 and 2 we therefore used the quotation from Sartorius. Cost estimates for the glass influent and effluent vessels are based on a quotation from Saillart (Belgium). Table 19 shows that the change in material strongly reduces the hardware costs. The different supply strategy has a limited impact on the assembly costs.

Table 19: Compartment II hardware and construction costs for 2 scenarios, assuming that Sartorius delivers the bioreactor with control unit. The automation alternatives are compared later in 4.3.6.

	scenario 1: steam sterilizable	**scenario 2: autoclavable**
hardware		
material	glass bioreactor stainless steel tanks stainless steel piping	glass bioreactor glass tanks plastic tubing
bioreactor + control	89 kEUR	56.5 EUR
influent + effluent vessels	202 kEUR	12 kEUR
sensors, transmitters, valves, filters, ..	included in above	32 kEUR
illumination system	15 kEUR (not included in Table 7)	1.3 kEUR
side equipment	35 kEUR	8.8 kEUR
total hardware	**341 kEUR**	**111 kEUR + 25 kEUR for steam sterilizable couplings**
assembly		
assembly by VITO	2 kEUR	5 kEUR
training	5 kEUR	5 kEUR
total	**348 kEUR**	**121 kEUR + 25 kEUR for steam sterilizable couplings**

Overall cost comparison

The time needed for autoclaving or steam sterilizing the influent and effluent tanks is considered to be similar. The main difference in operational costs is therefore related to the increased risk of contamination. As indicated before, the investment in steam sterilizable couplings is estimated to be 25 kEUR for 3 connections and 2 sampling ports, but is not an option because of safety risks. Additional operational costs for scenario 2 consist of tubing replacements at 150 m/yr. This amounts to 10 kEUR/yr. As shown in Table 20, scenario 2 without steam sterilizable connections and ports amounts to a total cost which is similar to scenario 1, when compartment operation over a period of > 8 years is considered.

Table 20: Cost comparison for 2 scenarios. The cost of external cooling units, steaming in place equipment, etc is not included in the price comparison.

	scenario 1: steam sterilizable hardware	**scenario 2: autoclavable hardware**	
		steam sterilizable connections and sampling ports	**no steam sterilizable connections and sampling ports**
hardware cost	341 kEUR (see Table 19)	136 kEUR	111 kEUR
assembly cost	7 kEUR	10 kEUR	10 kEUR
operation cost	reference scenario	reference scenario flexible tubing: 10 kEUR/yr	reference scenario restarting cultures: 20 kEUR/yr flexible tubing: 10 kEUR/yr
total	348 kEUR	146 kEUR (hardware) 10 kEUR/yr (tubing)	121 kEUR (hardware) 30 kEUR/yr (tubing + restarting)

- Adjustment side-equipment, illumination system and stirrer to 2 working volumes: the illumination system will be divided into 2 circuits. The lowest circuit will be needed for operation at the 5 l working volume, both will be needed for operation at 10 l. In both circuits, light intensity will be variable. When shifting from 5 to 10 l working volumes, all halogen lamps will be replaced to avoid that differences in intensity (decay) occur. The blades on the stirrer will be positioned in such a way that they provide optimal mixing for both working volumes. The extra costs for the adjustments on illumination and stirring are expected to be marginal.

- Biomass concentration sensor
  A biomass sensor becomes competitive with off-line analyses when experiments run longer than 2 years (Table 21), which is the case for BELISSIMA.

Table 21: Cost comparison on-line versus off-line biomass measurement.

	on-line +off-line analysis	**off-line analysis only**
type	sensor + dry weight	dry weight
frequency	1/week (TN80.16)	3/week + intensive sampling campaign to allow comparison reactor content and effluent
cost	sensor (turbidity) + transmitter: 5250 EUR off-line analysis: 26 EUR/week or ~1300 EUR/yr	78 EUR/week + 650 EUR for campaign ~4600 EUR/yr

- Conductivity sensor
  Taking into account the long-term activity in BELISSIMA, it is preferable to include a conductivity sensor (Table 22) compared to performing off-line analyses.

Table 22: Cost comparison on-line versus off-line conductivity measurement.

	on-line analysis	**off-line analysis**
type	sensor	dry weight
frequency	continuous (TN80.16)	5/week on effluent
cost	sensor + transmitter: 2500 EUR	35 EUR/week ~1750 EUR/yr

- Redox sensor: Either redox is measured on-line or it is not measured at all. In any case, a sampling port will be available to allow implementation of the sensor at a later stage, when this is deemed necessary. A redox sensor and transmitter cost 2200 EUR. At this stage a redox sensor is not considered necessary for process follow-up.

With the additional costs of 8 kEUR for sensors, scenario 2 hardware costs for compartment II would be in the order of 160 kEUR.

4.3.4.Compartment III

- Steam-sterilizable versus autoclavable hardware: Like for compartment II, the switch from steam sterilizable to autoclavable hardware has an impact on the risk of contamination as well as on the supply strategy and the assembly and operation of the compartment.

Contamination risk
Operation of compartment III consist of a series of actions which is similar to those described for compartment II. As a result, the main differences in contamination risks between both scenarios lie in the coupling of sterilized influent or effluent tanks to the bioreactor, the transfer of autoclaved medium to the sterilized influent tank and sampling from the effluent tank. Sampling from the bioreactor is not planned for compartment III because of the slow growth and very high risk of contamination of the nitrifying biomass.

The risk of contamination and the need to restart the loop will be significantly higher in scenario 2 than in 1. For compartment III, the impact will be much higher than in compartment II because the nitrifying coculture grows very slowly. The time needed for a restart is estimated to be 2 months. This corresponds to an additional cost of ~30 kEUR per event or an extra yearly cost of 60 kEUR for scenario 2 assuming 2 events per year compared to scenario 1.

Supply strategy and assembly

In scenario 1, a near-complete unit including influent and effluent tanks and piping is delivered by 1 supplier and VITO does not have to do any engineering work. The supplier quotations have been compared in Table 8. The cheapest supplier was Applikon with offers between 259 and 335 kEUR.

In scenario 2, the bioreactor is delivered by one supplier, whereas the influent and effluent tanks are provided by another one. VITO would then perform the final assembly. For the bioreactor system, a quotation is available from Sartorius only. Cost estimates for the glass influent and effluent vessels are based on a quotation from Saillart (Belgium). For the other parts, no quotations are available. They were therefore considered to be identical to compartment II, except for the bioreactor which is more complex for compartment III due to its fixed bed concept. Table 23 shows that the change in material strongly reduces the hardware costs. The different supply strategy has a limited impact on the assembly costs.

Table 23: Compartment III hardware and construction costs for 2 scenarios. The automation alternatives are compared later in 4.3.6.

	scenario 1: steam sterilizable		**scenario 2: autoclavable**
	Sartorius	**Applikon**	
hardware			
material	glass bioreactor stainless steel tanks stainless steel piping		glass bioreactor glass tanks plastic tubing
bioreactor + control	172 kEUR	not detailed	70 kEUR
influent + effluent vessels	221 kEUR	not detailed	12 kEUR
sensors, transmitters, valves, filters, ..	included in above		32 kEUR
side equipment	12 kEUR	not detailed	10 kEUR
total hardware	**405 kEUR**	**259-335 kEUR**	**124 kEUR**
assembly			
assembly by VITO	2 kEUR	2 kEUR	5 kEUR
training	7 kEUR	7 kEUR	7 kEUR
total	**414 kEUR**	**268-344 kEUR**	**136 kEUR**

Overall cost comparison

The time needed for autoclaving or steam sterilizing the hardware is considered to be similar. The only difference in operational costs would therefore be related to the increased risk of contamination. Table 24 then indicates that both scenarios are comparable in costs when long-term operation is taken into account.

Table 24: Cost comparison between scenario 2 and the cheapest Applikon option for scenario 1.

	scenario 1: steam sterilizable	scenario 2: autoclavable
hardware cost	268 kEUR (see Table 23)	136 kEUR
assembly cost	7 kEUR	12 kEUR
operation cost	reference scenario	reference cost flexible tubing (150 m/yr): 10 kEUR/yr
total	275 kEUR (hardware)	148 kEUR (hardware) + 10 kEUR/yr

4.3.5.Compartment IVa

- Steam-sterilizable versus autoclavable hardware: this compartment is highly similar to compartment II. We therefore refer to the discussion and cost comparison for compartment II.

- Adjust side-equipment, illumination system and stirrer to 2 working volumes: similar considerations apply as for compartment II. The extra costs are negligible.

- Biomass concentration sensor
  For timeframes longer than 1 year, the implementation of a biomass sensor is the cheapest solution (Table 25).

Table 25: Cost comparison on-line versus off-line biomass measurement.

	on-line +off-line analysis	off-line analysis only
type	sensor + dry weight	dry weight
frequency	3/week (TN80.16)	5/week + intensive sampling campaign to allow comparison reactor content and effluent
cost	sensor (turbidity) + transmitter: 5250 EUR off-line analysis: 78 EUR/week or ~4000 EUR/yr	130 EUR/week + 650 EUR for campaign ~7100 EUR/yr

4.3.6.Automation options

For the automation of the BELISSIMA loop, several options are available. For compartment I, EPAS was most experienced and proposed a PLC-based automation which would cost around 37 kEUR. For compartments CII to IVa, Sartorius and Applikon propose to use their local control systems. The only quotation available is from Sartorius and amounts to 20 kEUR per compartment. If compartments II to IVa were ordered from different suppliers, it is not clear to what extent their respective local control systems would be compatible.
Alternatively, VITO can harmonize the automation, which would then however have to be subcontracted. Based on a quotation received from the company Welec, the

automation costs were estimated for CII first and then for additional compartments (Table 26). In spite of cost reductions for electrical schemes and make up and servicing of software for additional compartments CIII and CIVa, the total costs are much higher than those proposed by Sartorius and EPAS.

Table 26: Cost comparison in kEUR for (subcontracted) automation of different BELISSIMA compartments. *When EPAS does the PLC programming for CI, this number can be reduced.

	CI	CII	CIII	CIVa
electrical schemes	7	6	3	3
electrical cabinet with components, cabled	25.7	20.7	20.7	20.7
delivery and installment PLC control	17.3	17.3	17.3	17.3
make up and servicing software	25*	22.4	11.2	11.2
SCADA supervision system		7.8		
industrial PC	2.5	2.5	2.5	2.5
total	**77.4**	**76.5**	**55.5**	**55.5**

It should further be noted that there is a need of harmonization of control strategy (loops, hardware, software) within the MELiSSA Project

4.4. Conclusions

From the analysis, it is clear that scenario 1 and 2 are comparable in terms of costs when the long-term perspective of the BELISSIMA study is taken into account. However, scenario 2 presents a higher contamination risk. Our proposal to reduce such risks by combining autoclavable hardware with steam sterilizable connections and sampling ports was not feasible because of safety risks. ESA and VITO therefore concluded that scenario 1 was preferable taking both costs and contamination risks into account. As hardware costs for steam sterilizable hardware were higher than originally budgeted, it was decided to revise the contents and budgets of the BELISSIMA I contract in line with scenario 1.
The detailed design for compartment I, described in 5.2, is therefore in accordance with the assumptions of scenario 1.

5. Recommended design

5.1. Waste preparation unit

5.1.1.Off-line kitchen cutter

The feed waste material to the BELISSIMA loop includes vegetables and toilet paper. Similar to the approach of the MELiSSA Pilot Plant, it is envisaged that this material be reduced in size in an off-line kitchen cutter before being introduced into the Waste Preparation Unit.
A survey for hygienic kitchen cutters showed that the company Robot Coupe (www.robot-coupe.be) provides such material for application in industrial kitchens, restaurants, hospitals, etc. The vertical cutters exist in various sizes and allow to process quantities between 23 and 60 l. The cutters are made in inox and are equipped with 2 or 3 cutting blades, respectively for the treatment of small or maximal amounts of material. For an optimal result, the distance between the blades can be properly adjusted. To process frozen material, the blades should have milled edges (Figure 5). The inox container in which the material has been cut, can be tilted for emptying and can be removed for easy cleaning.

Figure 5. Picture of vertical cutter (left), and close-up of milled edges (right).

VITO performed tests with lettuce, red beet and toilet paper at Robot Coupe premises in Mont-Sainte-Geneviève (Belgium). In a first test, frozen red beet, frozen lettuce and toilet paper were added sequentially and processed in the same batch (Figure 6). In a second test, the lettuce was processed first and then red beet was added (Figure 7). The results looked satisfying in that a homogeneous mixture was obtained of small particle size. It is therefore proposed to purchase a Robot Coupe vertical cutter for the first preparation step of the frozen vegetables and toilet paper.

Figure 6. Cutting result (right) after sequential addition of red beet, lettuce and toilet paper (left) to the same batch.

Figure 7. Cutting result (right) after sequential addition of lettuce and red beet (left).

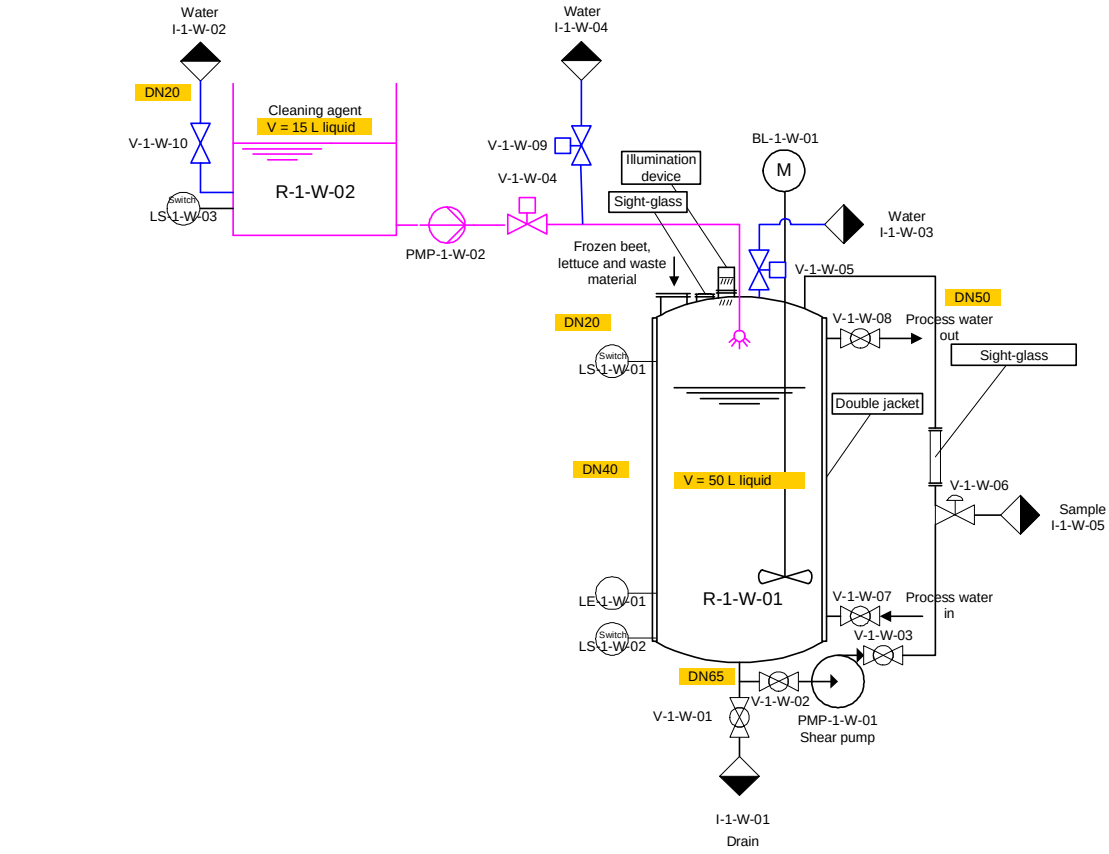
5.1.2.Description of actual Waste Preparation Unit

Frozen beet and lettuce and toilet paper will be reduced in size by an off-line kitchen cutter (see 5.1.1). They are then transferred into tank R-1-W-01 through an opening in the top lid. Wheat straw is ground separately in 2 steps with two grinders from Retsch and sieved at 2 mm (tested before by EPAS and UAB), before being introduced in tank R-1-W-01. Then, the frozen urine and fecal material will be added directly into tank R-1-W-01. The suspension is then diluted to the desired concentration through the addition of (distilled) water. While the waste is circulated in a loop over R-1-W-01, further particle size reduction is achieved through the in-line pump PMP-1-W-01. The selected pump (Fristam) was tested at UAB, albeit as a lower power version. The 11 kW version chosen here, is expected to perform better, but has not been tested yet.

A sight-glass in the recirculation line allows to evaluate the mixing process. The final waste material can also be sampled through I-1-W-05. Through the same interface and using PMP-1-W-01, the material can be transferred to the influent tank of Compartment I. The loop can be drained through an outlet at the bottom of R-1-W-01. Tank R-1-W-01 must be able to contain the total influent for at least 10 days. Although in principle a maximal flow of 2 l/d is envisaged, its volume is proposed to be 50 l and 40 l batches will be processed each time. It will be equipped with a sight-glass in the top lid and can be illuminated. It has a double jacket. In case substantial temperature increases occur during mixing, this will allow for a future implementation of cooling liquid for temperature control. This is however not required at this stage.

The feed preparation unit will be cleaned after each use. To this end, an additional tank R-1-W-03 is provided from which flush water + cleaning agent can be pumped (PMP-1-W-02) into R-1-W-01 and R-1-W-02 through nozzles at the top of the tanks and also in the lower part of R-1-W-01. The flush water can be recycled with PMP-1-W-01 and drained through opening of valve V-1-W-01. Direct addition of flush water from a tap is also possible through V-1-W-11.
Because of the foam formation in R-1-W-01, level switch LS-1-W-01 is adapted to detect foam and liquid. Level detection is hydrostatic (LE-1-W-01). Other level switches are classical level switches.

5.1.3.P&ID



EPAS n.v. De Prijkels - Venecoweg 19 B-9810 Nazareth Tel: +32 9/381.51.30 Fax: +32 9/221.82.18 epas@epas.be	DRAWING TITLE **BELISSIMA Waste Preparation Unit**	CLIENT **VITO**
	DRAWN BY Jan Stuyck/Helmut Elslander	DATE 11-03-09
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Figure 8. Waste preparation unit

5.1.4.Component list

Table 27. Component list for feeding preparation

Tag	Purpose - Description	Supplier	Ref. / order n°
Waste Preparation unit			
LS-1-W-01	Level switch (foam detection)	E+H www.endress.com	Liquiphant M FTL51H, SS compact housing, ISO 2852 Tri-Clamp 1 ½" process connection, SS 316L.
LS-1-W-02	Level switch	E+H	Liquiphant T FTL20H, SS compact housing, ISO 2852 Tri-Clamp 1 ½" process connection; SS 316L.
LS-1-W-03	Level switch	E+H	Liquiphant T FTL20H, SS compact housing, ISO 2852 Tri-Clamp 1 ½" process, SS 316L.
LE-1-W-01	Level transmitter	E+H	Levelflex M FMP41C or Deltapilot S FMB70, SS housing, ISO 2852 Tri-Clamp 1 ½" process, SS 316L
PMP-1-W-01	Shear pump	Fristam www.fristam.de	FSPE 3522/145 A, rotor 145 mm, 11 kW, Cr-Ni-Mo steel 1.4404, with frequency drive
PMP-1-W-02	Magnetic drive centrifugal pump	Iwaki www.iwaki.be	Type YMD, SS 316L.
R-1-W-01 and BL-1-W-01	Buffer tank, double jacket, stainless steel, 50 l Removable top with sight glass, illumination device, opening (min 200 mm) for waste material addition and spare hygienic port. Equipped with spray ball for tank cleaning and (magnetically coupled) stirrer at variable frequency. Equipped with sanitary connections to instrumentation and devices for easy cleaning.	www.nocado.de, www.prg-gmbh.de	Tank: SS316L, inside 0,8 µm, outside 1,6 µm; Sight-glass + illumination device: Nocado, Nocaplus SS 316L Ra 0,8 µm; Removable top: Nocado, Nocaplus SS 316L Ra 0,8 µm Spray balls: Nocado, Nocaplus SS316L Ra 0,8 µm; Stirrer: PRG (to be specified), with frequency drive Stirrer type and drive depend on tank dimension and medium characteristics
R-1-W-02	Cleaning tank, 15 l		SS 316 L, inside Ra 0,8 µm
V-1-W-01	Manual 2-way ball valve (drain buffer tank)	Nocado www.nocado.de	Nocaplus SS 316L, DN (to be specified), Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-02	Manual 2-way ball valve	Nocado	Nocaplus SS 316L, DN (to be specified), Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-03	Manual 2-way ball valve	Nocado	Nocaplus SS 316L, DN (to be specified), Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-04	Powered 2/2 way diaphragm valve	Nocado	Nocaplus SS 316L, DN 15, diaphragm material: to be specified, Ra 0,8

Tag	Purpose - Description	Supplier	Ref. / order n°
			µm, Tri-Clamp process connection.
V-1-W-05	Powered 2/2 way diaphragm valve	Nocado	Nocaplus SS 316L, DN 25, diaphragm material: to be specified, Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-06	Manual 2 way diaphragm valve	Nocado	Nocaplus SS 316L, DN 15, diaphragm material: to be specified, Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-07	Manual 2-way ball valve	Nocado	Nocaplus SS 316L, DN (to be specified), Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-08	Manual 2-way ball valve	Nocado	Nocaplus SS 316L, DN (to be specified), Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-09	Powered 2/2 way diaphragm valve	Nocado	Nocaplus SS 316L, DN 15, diaphragm material: to be specified, Ra 0,8 µm, Tri-Clamp process connection.
V-1-W-10	Manual 2-way ball valve	Nocado	Nocaplus SS 316L, DN 25, Ra 0,8 µm, Tri-Clamp process connection.
I-1-W-01	Drain (tubing)		
I-1-W-02	Inlet for water (connection to tap or distilled water)		
I-1-W-03	Inlet for water (connection to tap or distilled water)		
I-1-W-04	Inlet for water (connection to tap or distilled water)		
I-1-W-05	Inlet for water (connection to tap or distilled water)		
I-1-W-06	Sampling or transfer to influent tank compartment I		
Wheat straw preparation			
	Grinder for raw grinding: Cutting mill SM100	Retsch	
	Grinder for thin grinding: UltraCentrifugal Mill ZM200	Retsch	
Vegetable preparation			
	Vertical cutter R30	Robot Coupe	

All components of the waste preparation unit that have analogue outputs, inputs, digital outputs and/or inputs are listed in Table 28.

Table 28. Electro-technical characteristics

Tag number	Description	Voltage (V)	AO	AI	DO	DI
LS-1-W-01	Level switch (foam detection)	24VDC			1	
LS-1-W-02	Level switch	24VDC			1	
LS-1-W-03	Level switch	24VDC			1	
PMP-1-W-01	Shear pump	230-400VAC/3Ph				
PMP-1-W-02	Centrifugal pump	230VAC				
V-1-W-04	Powered 2/2 way valve	24VDC				1
V-1-W-05	Powered 2/2 way valve	24VDC				1
V-1-W-09	Powered 2/2 way valve	24VDC				1

5.1.5.PLC IO list

Tag number	Description	Voltage (V)	AI	AO	DI	DO
General						
	Emergency Stop	24VDC			1	
	Switch for activation of PMP-1-W-01	24VDC			1	
	Switch to clean R-1-W-01	24VDC			1	
	Switch to fill R-1-W-01 with water	24VDC			1	
Waste preparation unit						
LS-1-W-01	Level switch (foam detection)	24VDC			1	
LS-1-W-02	Level switch	24VDC			1	
LS-1-W-03	Level switch	24VDC			1	
PMP-1-W-01	Shear pump	230-400VAC/3Ph				1
PMP-1-W-02	Centrifugal pump	230VAC				1
V-1-W-04	Powered 2/2 way valve	24VDC				1
V-1-W-05	Powered 2/2 way valve	24VDC				1
V-1-W-09	Powered 2/2 way valve	24VDC				1

5.2. Compartment I

The process is an anaerobic process that takes place in a cylindrical bioreactor at 55°C. The bioreactor is fed with influent (a mix of faecal material, urine, water, toilet paper and plants) semi-continuously by an influent pump. The influent is stored in an influent buffer tank which is cooled to prevent that the degradation starts in the buffer. The anaerobic process is realized by bacteria autochthonous from the waste. An external tangential filtration unit allows to continuously separate the soluble components from the bacteria and the solid particles which need more time to be degraded. The soluble components are harvested in a sterile filtrate tank.

5.2.1.Hardware description

The set-up for compartment I consists roughly of an influent tank, an anaerobic reactor, a filtration unit and a filtrate tank. Within this paragraph, the hardware is described in detail. More information on the exact operation of this compartment can be found in 5.2.5.

5.2.1.1. *Influent tank*

The influent tank is a standard stainless steel reactor with a double-jacket (volume ~20L). The influent is cooled using a cooler connected to the double-jacket. It is continuously mixed with a blender. The temperature is measured on-line for an accurate temperature control. The influent is continuously circulated through a loop using a lobe pump (P-1-I-01) to prevent clogging and to homogenize the influent. At regular time intervals, the automated 3 way valve P3V-1-I-01 switches position and opens in the direction of the bioreactor, so that it is fed in a semi-continuous mode (~2 L/d).

The lobe pump P-1-I-01 recirculates the feed mixture over the influent tank. When the valve head is open, it creates a short cut between discharge and suction side of the pump. Due to the large size of the valve head, the full capacity of the pump can pass through the valve from the discharge back to the suction side. This way, with the correct setting, it is not possible to overpressurize the pump. The valve is easy to clean or check and its head has been designed to maximize the flow passage section and to minimize pressure losses as well as to allow particles to pass through. The differential pressure on the pump is influencing the load that is acting on the valve head. The set value of the spring or air pressure is balancing the valve head. When the differential pressure of the pump becomes higher than the valve settings, the valve head will open. If the pump is working against a closed discharge valve, the medium circulates inside the pump via the relief valve. The hydraulic power and the friction losses are transformed to thermal energy and the temperature of this relatively small volume of circulating fluid will rise if the pump continues to operate for an extended period of time. In severe cases this may result in temperatures exceeding the operating limits of the pump or in vaporization of the fluid, both of which should be avoided.

The different hardware items are listed in Table 29.

Table 29 . Influent tank hardware

Tag	Purpose/description	Supplier	ref./order n°
VSL2-1-I-01 with BLE-1-I-01	Influent buffer, double jacket, stainless steel, 20 l Inspection opening, spare hygienic port. Equipped with spray ball for tank cleaning and (magnetically coupled) stirrer at variable frequency. Equipped with sanitary connections to instrumentation and devices for easy cleaning.		Tank: SS316L, inside 0,8 µm, outside 1,6 µm; Spray balls: see CIP, SIP Stirrer: to be specified, with frequency drive Stirrer type and drive depend on tank dimension and medium characteristics
PT-1-I-01	Level transmitter influent tank (level measurement based on differential pressure to PT-1-I-02)	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-I-02	Pressure transmitter	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-I-03	Pressure transmitter after pump P-1-I-01	E+H	Cerabar T: PMP135, Ra 0,8 µm, Tri-Clamp process connection
LS-1-I-01	Level switch HH	E+H	Liquiphant T FTL20H, Ra 0,8 µm, Tri-Clamp process connection
LS-1-I-02	Level switch LL	E+H	Liquiphant T FTL20H, Ra 0,8 µm, Tri-Clamp process connection
TT-1-I-01	Temperature transmitter	E+H	Omnigrad M TR45, Ra 0,8 µm, Tri-Clamp process connection
P-1-I-01	Recirculation pump influent loop with frequency drive	Johnson Pumps	Johnson CP10/005/12 (Lobe pump, 20l/h, 32rpm, rotor, axe, rotor house, etc. RVS316, sealing Viton, 1" Tri-clamp connections, multilobe rotor form, 0,37kW, 400V 3ph Includes overpressure relief valve
RV-1-I-01	Pressure Relief Valve	LESER	Type 483
P3V-1-I-01	Powered 3-way Koltek Shutter valve, pneumatic actuated	Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection and position indication "ThinkTop"
HV-1-I-01	Manual 2-way Sanitary Ball Valve on top of tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-I-02	Manual 2 way valve on top of tank	Swagelok	SS 43G-Series, 6 mm
HV-1-I-03	Manual 2-way Sanitary Ball Valve to pump P-1-F-01	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-I-04	Manual 2-way Sanitary Ball Valve after pump P-1-F-01	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-I-05	Manual Sanitary Tank Bottom Sample Valve	Nocado, Alfa Laval	SS 316L, sampling port ≥ 10 mm , Ra 0,8 µm, Tri-Clamp process connection.
HV-1-I-06	Manual 2 way valve, gas supply to influent tank	Swagelok	SS 43G-Series, 6 mm
HV-1-I-07	Manual 2-way Sanitary Ball Valve, cooling water to influent tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-I-08	Manual 2-way Sanitary Ball Valve, cooling water to	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection

	cooler		
HV-1-I-09	Manual 2 way valve, on top of influent tank	Swagelok	SS 43G-Series
HV-1-I-10	Manual 2-way Sanitary Ball Valve, drain	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HX-1-I-01	Cooler for influent tank	Huber	Unichiller UC017Tw (1.7 kW at 15°C), equipped with controller "Professional"

5.2.1.2. *Bioreactor*

The bioreactor will be a stainless steel reactor with double-jacket.
The bioreactor temperature (55°C) is controlled based on a temperature sensor and with a heat exchanger coupled to the double jacket. It is continuously mixed with a blender. The pH is measured on-line by one sensor. Based on the pH measurement, acid or base are automatically added to the bioreactor for pH correction. They are stored in glass bottles and fed with small electromagnetic metering pumps. The amount dosed will be monitored with balances. The pressure is kept constant in the bioreactor and the volume is determined by on-line pressure sensors. The bioreactor can be operated at 12 l or at 19 l depending on required sampling volumes (see also Figure 2).
Level measurement for volume control is based on differential pressure PT-1-B-01 – PT-1-B-02, which is independent of gas production as long as the pressure remains within the range of the pressure transducers. Two different sampling ports at different heights allow to check homogeneity of the bioreactor content.

The different hardware items are listed in Table 30.

Table 30. Bioreactor hardware

Tag	Purpose/description	Supplier	ref./order n°
VSL2-1-B-01 with BLE-1-B-01	Bioreactor, double jacket, stainless steel, 19 l Inspection opening, spare hygienic port. Equipped with spray ball for tank cleaning and (magnetically coupled) stirrer at variable frequency. Equipped with sanitary connections to instrumentation and devices for easy cleaning.		Tank: SS316L, inside 0,8 µm, outside 1,6 µm; Spray balls: see CIP, SIP Stirrer: to be specified, with frequency drive Stirrer type and drive depend on tank dimension and medium characteristics
PT-1-B-01	Level transmitter (level measurement based on differential pressure to PT-1-B-02)	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-B-02	Pressure transmitter	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
LS-1-B-01	Level switch HH	E+H	Liquiphant T FTL20H, Ra 0,8 µm, Tri-Clamp process connection
LS-1-B-02	Level switch LL	E+H	Liquiphant T FTL20H, Ra 0,8 µm, Tri-Clamp process connection
TT-1-B-01	Temperature transmitter	E+H	Omnigrad M TR45, Ra 0,8 µm, Tri-Clamp process connection
AIT-1-B-01	pH measurement	Knick, Mettler Toledo	pH transmitter Knick Pro in combination with InPro 3250 pH electrode (standard KCl electrode) and InFit 762e insertion housing with Tri- Clamp process connection **or** pH transmitter Knick Pro in combination with InPro 2000 (refillable electrode with silver-ion trap to prevent contamination and clogging of the diaphragm in sulphide containing process medium) and InFit 763e insertion housing with Tri-Clamp process connection.
RV-1-B-01	Pressure Relief Valve	LESER	Type 483
HV-1-B-01	Manual 2-way Sanitary Ball Valve, to sampling on top of bioreactor	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-02	Manual 2-way Sanitary Ball Valve, influent feeding	Nocado, Alfa	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process

		Laval	connection
HV-1-B-03	Manual 2-way Sanitary Ball Valve, return retentate filtration	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-04	Manual 2-way Sanitary Ball Valve, recirculation permeate to bioreactor	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-05	Manual 2-way Sanitary Ball Valve, medium to filtration unit	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-06	Manual Sanitary Tank Bottom Sample Valve	Nocado, Alfa Laval	SS 316L, sampling port ≥ 10 mm , Ra 0,8 µm, Tri-Clamp process connection.
HV-1-B-07	Manual 2 way valve, gas supply to bioreactor	Swagelok	SS 43G-Series, 6 mm
HV-1-B-08	Manual 2-way Sanitary Ball Valve, water to double jacket	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-09	Manual Sanitary Tank Sample Valve	Nocado, Alfa Laval	SS 316L, sampling port ≥ 10 mm , Ra 0,8 µm, Tri-Clamp process connection.
HV-1-B-10	Manual Sanitary Tank Sample Valve	Nocado, Alfa Laval	SS 316L, sampling port ≥ 10 mm , Ra 0,8 µm, Tri-Clamp process connection.
HV-1-B-11	Manual 2-way Sanitary Ball Valve, water to heat exchanger	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-12	Manual 2-way Sanitary Ball Valve, drain	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-B-13	Manual 2 way valve, to gas volume measurement	Swagelok	SS 43G-Series, 6 mm
HV-1-B-14	Manual 2 way valve, return condensate	Swagelok	SS 43G-Series, 6 mm
HV-1-B-15	Manual 2 way valve, to closed gas loop	Swagelok	SS 43G-Series, 6 mm
HV-1-B-16	Manual 2 way valve, return condensate	Swagelok	SS 43G-Series, 6 mm
HV-1-B-17	Manual 2 way valve, return closed gas loop	Swagelok	SS 43G-Series, 6 mm
HV-1-B-18	Manual 2 way valve, return condensate	Swagelok	SS 43G-Series, 6 mm
HX-1-B-01	Heat exchanger bioreactor	Huber	Unistat 405w (1,3 kW at 100°C), equipped with controller “Professional”
VSSL-1-C-01	Glass bottle	Duran Group	10 l
VSSL-1-C-02	Glass bottle	Duran Group	10 l
WIT-1-C-01	Balance	Mettler Toledo	Excellence XS Series, RS232 interface and optional Ethernet,

WIT-1-C-02	Balance	Mettler Toledo	Excellence XS Series, RS232 interface and optional Ethernet
P-1-C-01	Electromagnetic metering pump	Iwaki	EWF11TC-20EPY2, PVDF
P-1-C-02	Electromagnetic metering pump	Iwaki	EWF11TC-20EPY2, PVDF
NRV-1-C-01	Foot valve	Iwaki	FSTC-4x6, PVDF
NRV-1-C-02	Foot valve	Iwaki	FSTC-4x6, PVDF
NRV-1-C-03	Check valve	Iwaki	CCA-1FC-4x6, PVDF
NRV-1-C-04	Check valve	Iwaki	CCA-1FC-4x6, PVDF
HV-1-C-01	Manual 2 way valve	EM-Technik	Series 6L, PVDF, 6mm
HV-1-C-02	Manual 2 way valve	EM-Technik	Series 6L, PVDF, 6mm
HV-1-C-03	Manual 2 way valve	EM-Technik	Series 6L, PVDF, 6mm
HV-1-C-04	Manual 2 way valve	EM-Technik	Series 6L, PVDF, 6mm

5.2.1.3. *Gas Loop*

The Gas Loop is aimed to control and stabilize the pressure in the bioreactor, to accomplish a flow of gas through a non destructive on-line gas analyser and to evacuate and measure the produced biogas.

The overpressure in the bioreactor is kept constant at 100 mbar. When the pressure is decreasing, N_2 gas is automatically added using a pressure regulating valve (HPCV-1-G-06). When the pressure is increasing due to biogas production, the backpressure regulating valve HPCV-1-G-02 opens and lets the gas escape to the Milligas counter (FQI-1-G-01). The gas flow is cooled in a compressor cooler (HX-1-G-01) and the condensed water recycled to the bioreactor via valve HV-1-B-13. To avoid condensation in the piping from the bioreactor to the cooler, a heat trace is foreseen. The Milligas gas volume measurement is based on the following principle. The measurement cell consists of two chambers in which the filling liquid is gradually displaced by produced gas are filled consecutively by rising gas bubbles, which enter the system through a microcapillary at the bottom. When one chamber is filled with gas, the cell switches position through which the filled chamber is emptied and the second chamber is now filled with gas. Volume measurement is based on counting the number of switches. The Milligas counter will be adapted to a maximal overpressure of 100 mbar and gas volume measurement will be corrected for temperature and pressure, measured at the exit of the Milligas counter. To avoid backflow of the filling liquid to the bioreactor due to accidental underpressure, an extra separator is included.

An additional loop recirculates a gas flow with a rate of around 0,5 to 2 l/min through a gas analyser AIT-1-G-01. This flow is accomplished by a pump which is integrated in the analyser. The flow rate is controlled by the analyser but an additional indicator FI-1-G-01 is provided. The analyser cannot handle liquid and therefore the gas flow from and to the bioreactor is dehumidified by compressor cooler HX-1-G-01. Afterwards it is heated by tracing HX-1-G-04. A combined filter GF-1-G-01 with integrated liquid sensor removes any remaining liquid. Valves HV-1-G-01 and -02 allow calibration of the analyser with N_2 and a calibration gas mixture. Flow meters FI-1-G-02 and -03 ensure surplus of calibration gas flow of which a (manually controlled) partial stream is sent to the analyser.

The gas cooler HX-1-G-01 can handle three separate lines of gas and is used to dehumidify both the gas for gas volume measurement and the gas in the analyser loop.

The different hardware items are listed in Table 31.

Table 31. Gas loop hardware

Tag	Purpose/description	Supplier	ref./order n°
HX-1-G-01	Sample Gas Conditioner	AGT Thermotechnik	MAK10-3 (optional externally installed moisture sensor).
HX-1-G-02	Heated hose with integrated temperature sensor TE-1-G-01, controller TIC-1-G-01 and exchangeable inner tube	Winkler	WAP 106/ 04, Pt 100, controller JUMO iTRON DR 100
HX-1-G-03	Heated hose with integrated temperature sensor TE-1-G-02, controller TIC-1-G-02 and exchangeable inner tube	Winkler	WAP 106/ 04, Pt 100, controller JUMO iTRON DR 100
HX-1-G-04	Heated hose with integrated temperature sensor TE-1-G-03, controller TIC-1-G-03 and exchangeable inner tube	Winkler	WAP 106/ 04, Pt 100, controller JUMO iTRON DR 100
SEP- 1-G-01	Liquid Separator	Ankersmid	ACS- P/D
FQI-1-G-01	Gas Counter	Ritter	MilliGascounter type MGC-1
FQT-1-G-01	Pulse transmitter	Ritter	Pulse conversion to 4-20 mA type EDU 32 FP
PT-1-G-01	Pressure transmitter	Ritter	BnC-Ritter, Druck transducer
TT-1-G-01	Temperature transmitter	Ritter	BnC-Ritter, Thermometer
GF-1-G-01	Aerosol, particle filter and water stop (hydrophobic filter with integrated liquid alarm sensor ME-1-G-01	Ankersmid	AFP 01 filter, ALA 002 liquid alarm sensor
FI-1-G-01	Variable Area Flowmeter with needle valve	KROHNE	DK47, SS, 0-100Nl/h
FI-1-G-02	Variable Area Flowmeter with needle valve	KROHNE	DK47, SS, 0-100Nl/h
FI-1-G-03	Variable Area Flowmeter with needle valve	KROHNE	DK47, SS, 0-100Nl/h
AIT-1-G-01	CO_2/CH_4 IR Gas Analyzer with integrated sample gas pump, flow sensor en humidity sensor.	Sick/Maihak	SIDOR. Measuring ranges to be specified.
HPCV-1-G-01	Manual Backpressure Regulator with integrated pressure indicator PI-1-G-01, on influent tank	Swagelok	KBP (0-0,680 barg), SS
HPCV-1-G-02	Manual Backpressure Regulator with integrated pressure indicator PI-1-G-02 on bioreactor	Swagelok	KBP (0-0,680 barg), SS
HPCV-1-G-03	Manual Pressure regulator with integrated pressure indicator PI-1-G-03 in N2 line	Swagelok	KPR (0-0,680 barg), SS

HPCV-1-G-04	Manual Pressure regulator with integrated pressure indicator PI-1-G-04 in line with calibration gases	Swagelok	KPR (0-0,680 barg), SS
HPCV-1-G-05	Manual Pressure regulator with integrated pressure indicator PI-1-G-05 to influent tank headspace	Swagelok	KPR (0-0,680 barg), SS
HPCV-1-G-06	Manual Pressure regulator with integrated pressure indicator PI-1-G-06 to bioreactor headspace	Swagelok	KPR (0-0,680 barg), SS
HPCV-1-G-07	Manual Pressure regulator with integrated pressure indicator PI-1-G-07 to filtration unit	Tescom	44-3262, SS, 0-6,8 barg
HPCV-1-G-08	Manual Pressure regulator with integrated pressure indicator PI-1-G-08 to filtration unit	Tescom	44-3261, SS, 0-3,5 barg
HPCV-1-G-09	Manual Pressure regulator with integrated pressure indicator PI-1-G-09 to permeate tank	Tescom	44-3262, SS, 0-6,8 barg
HPCV-1-G-10	Manual Pressure regulator with integrated pressure indicator PI-1-G-09 to permeate tank	Tescom	44-3261, SS, 0-3,5 barg
HPCV-1-G-11	Manual Backpressure Regulator with integrated pressure indicator PI-1-G-01 on permeate tank	Tescom	44-2360, SS, 0-1,7 barg
HV-1-G-01	Manual 2 way valve	Swagelok	SS 43G-Series, 6 mm
HV-1-G-02	Manual 2 way valve	Swagelok	SS 43G-Series, 6 mm
HV-1-G-03	Manual 2 way valve before gas analyser	Swagelok	SS 43G-Series, 6 mm
HV-1-G-04	Manual 2 way valve to exhaust in gas recirculation line	Swagelok	SS 43G-Series, 6 mm
HV-1-G-05	Manual 2 way valve	Swagelok	SS 43G-Series, 6 mm
HV-1-G-06	Manual 2 way valve	Swagelok	SS 43G-Series, 6 mm
SDV-1-G-01	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated to filter GF-1-F-01	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-G-02	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated to filter GF-1-F-02	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49

SDV-1-G-03	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated to filter GF-1-F-02	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces With position indication SK49
HDV-1-G-01	Manual 2-way Sanitary Diaphragm Valve to filter GF-1-F-01	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; Handwheel: PA6GF30

5.2.1.4. *Filtration unit (FU)*

The main views of the FU operation are presented in paragraph 5.2.3.

In filtration mode (Figure 10), the mixed liquor is continuously circulated from the bioreactor over one ceramic membrane UF-1-F-01 through a retentate loop back to the bioreactor. To this end, a lobe pump (P-1-F-01) is used which can operate at crossflows up to 5 m/s. The characteristics of this pump are similar to the ones of P-1-I-01 (5.2.1.1). A constant flow of filtrate is permeated through the membrane with a magnetic coupled pump P-1-F-02, and stored in the effluent vessel VSL2-1-F-01. The dead volume of the filtration loop should be sufficiently small compared to the bioreactor volume. A threshold of 10% of the bioreactor volume at maximal working volume and 20% at minimal working volume is proposed. This implies a dead volume of 2 l at maximum. During manufacturing of CI specific attention will have to be paid to this dead volume. Contents of the membrane lumen is about 0,02 l. The internal volume of the proposed pump P-1-F-01 is 0.694 l. This means that 1,266 l remains for piping, connections and valves. If we make an estimation for 1 l dead volume in DN 25 piping (internal diameter 25 mm) this represents about 3.9 m.

In case the membrane needs to be replaced or cleaned, recirculation is switched to a parallel by-pass by switching valves P3V-1-F-01 and -02 (Figure 12) and permeate extraction is interrupted. The bypass mode is also activated when certain alarm situations occur, e.g. membrane clogging.

When the effluent vessel is full and in certain alarm situations, e.g. obstructions at the pressure side of P-1-F-02, filtration can be continued in recycle mode. In this condition, the full permeate flow is recycled back to the bioreactor by opening valve SDV-1-F-09 and closing SDV-1-F-15.

To reduce the number of entries into the bioreactor, the return flows of permeate and concentrate are connected to the influent feeding line before they enter the bioreactor.

The proposed membrane was selected in a comparative study performed by Technomembranes. It is a ceramic Kerasep membrane with internal diameter of 6 mm. The size reduction from 25 mm internal diameter of the piping to 6 mm in the membranes will have to be performed gradually to avoid clogging.

A spare membrane module and a separate loop for membrane cleaning are available to avoid long interruptions of compartment I operation due to cleaning or rinsing.

The effluent tank is cooled at 4°C through a double jacket and allows storage of permeate over several days. It is kept at a constant overpressure of 100 mbar.

In the recirculation line from bioreactor over membrane back to bioreactor, flow and temperature are measured as well as the pressure at the inlet and outlet of the membrane module. In the permeate line, an additional pressure measurement is provided to allow calculation of the transmembrane pressure.

Both CIP and SIP of the membrane, and the permeate line are possible (Figure 12 & Figure 14). This is explained in more detail in 5.2.1.5 and 5.2.1.6.

The different hardware items are listed in Table 32.

Table 32. Filtration unit hardware

Tag	Purpose/description	Supplier	ref./order n°
VSL2-1-F-01	Bioreactor, double jacket, stainless steel, 15 l Spare hygienic port. Equipped with spray ball for tank cleaning Equipped with Millipore Novaseptic sanitary connections to instrumentation and devices for easy cleaning.		Tank: SS316L, inside 0,8 µm, outside 1,6 µm;
VSSL-1-F-01	Vessel to collect cooling liquid during steam sterilization		Volume vessel > double jacket VSL2-1-F-01
UF-1-F-01	Membrane module	Novasep	Kerasep, 6 mm ID, 40 cm long, 75 cm², Zirconia layer, cutoff 300 kD, cross-flow target = 2 m/s
LF-1-F-01	Liquid filter with low product hold up design	Domnick Hunter	Filter Housing, in-line: type ZVDICE-B-BTB-B-E, SS, Ra < 0,8 µm, Tri-Clamp process connection; Filter Element: TETPOR LIQUID type ZHFT-BZ, 0,2 µm
LF-1-F-02	Liquid filter with low product hold up design	Domnick Hunter	Filter Housing, in-line: type ZVDICE-B-BTB-B-E, SS, Ra < 0,8 µm, Tri-Clamp process connection; Filter Element: TETPOR LIQUID type ZHFT-BZ, PTFE, 0,2 µm
GF-1-F-01	Gas filter	Domnick Hunter	Filter Housing, in-line: type ZVDICE-B-BTB-B-E, SS, Ra < 0,8 µm, Tri-Clamp process connection; Filter Element: HIGH FLOW TETPOR type ZCMTB-020Z-PE, PTFE 0,2 µm
GF-1-F-02	Gas filter	Domnick Hunter	Filter Housing, in-line: type ZVDICE-B-BTB-B-E, SS, Ra < 0,8 µm, Tri-Clamp process connection; Filter Element: HIGH FLOW TETPOR type ZCMTB-020Z-PE, PTFE 0,2 µm
GF-1-F-03			
PT-1-F-01	Pressure transmitter after pump P-1-F-01	E+H	Cerabar T: PMP135, Ra 0,8 µm, Tri-Clamp process connection
PT-1-F-02	Pressure transmitter after membrane module	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-F-03	Pressure transmitter before membranen module	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-F-04	Pressure transmitter at permeate side	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-F-05	Pressure transmitter after pump P-1-F-02	E+H	Cerabar T: PMP135, Ra 0,8 µm, Tri-Clamp process connection
PT-1-F-06	Pressure transmitter on permeate tank	E+H	Cerabar M: PMP45, Ra 0,8 µm, Tri-Clamp process connection
PT-1-F-07	Pressure transmitter in cleaning solution line to permeate tank	E+H	Cerabar T: PMP135, Ra 0,8 µm, Tri-Clamp process connection

LT-1-F-01	Level transmitter	VEGA	VEGAFLEX 63 (Guided Radar), internal microwave principle, rod 6 mm, Tri-Clamp process connection
TT-1-F-01	Temperature transmitter in retentate line membrane	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-F-02	Temperature transmitter in effluent tank	E+H	Omnigrad M TR45, Ra 0,8 µm, Tri-Clamp process connection
FIT-1-F-01	Flow transmitter	E+H	Promag 53H, DN 15, Ra 0,8 µm, Tri-Clamp process connection
P-1-F-01	Retentate pump with frequency drive	Johnson Pumps	Johnson CP30/0069/12 lobe pump. (1,15m³/h SST316L, Viton elastomers, 113rpm, 1,1kW 400V 3ph) Includes overpressure relief valve
P-1-F-02	Permeate pump with frequency drive	GATHER Industrie	GATHER B1 1,5-12, magnetic coupled and frequency drive (capacity 0,02-5,00 l/h). With bypass for steam sterilisation.
RV-1-F-01	Pressure Relief Valve	LESER	Type 484, Superior Cleanability
H3V-1-F-01	Manual 3-way Koltek Shutter valve before pump P-1-F-01	Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
H3V-1-F-02	Manual 3-way Koltek Shutter valve after pump P-1-F-01	Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
H3V-1-F-03	Manual 3-way Sanitary Ball Valve in cooling line permeate tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
H3V-1-F-04	Manual 3-way Sanitary Ball Valve in cooling line permeate tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-F-01	Manual 2-way Sanitary Ball Valve in cooling line permeate tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
HV-1-F-02	Manual 2-way Sanitary Ball Valve in cooling line permeate tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection
P3V-1-F-01	Powered 3-way Koltek Shutter valve, pneumatic actuated in bypass line membrane	Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection and position indication “ThinkTop”
P3V-1-F-02	Powered 3-way Koltek Shutter valve, pneumatic actuated in bypass line membrane	Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm, Tri-Clamp process connection and position indication “ThinkTop”
PV-1-F-01	Powered 2-way Ball Valve, pneumatic actuated to waste	Swagelok	SS 43G-Series, 8 mm; with position indication
PV-1-F-02	Powered 2-way Ball Valve, pneumatic actuated to waste	Swagelok	SS 43G-Series, 8 mm; with position indication
PV-1-F-03	Powered 2-way Ball Valve, pneumatic actuated to waste	Swagelok	SS 43G-Series, 8 mm; with position indication
PV-1-F-04	Powered 2-way Ball Valve, pneumatic actuated to waste	Swagelok	SS 43G-Series, 8 mm; with position indication
PV-1-F-05	Powered 2-way Ball Valve, pneumatic actuated to waste	Swagelok	SS 43G-Series, 8 mm; with position indication
PV-1-F-06	Powered 2-way Ball Valve, pneumatic actuated to waste	Swagelok	SS 43G-Series, 8 mm; with position indication

SDV-1-F-01 SDV-1-F-02 SDV-1-F-03	Powered 3 x 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated, left above membrane	KSB	Type SISTO-CM3XX DN25 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-04	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated, above membrane	KSB	Type SISTO-CAS DN25 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces;
SDV-1-F-05	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated, below membrane	KSB	Type SISTO-CAS DN25 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-06	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated, below membrane	KSB	Type SISTO-C DN25 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-07	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated, below membrane	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-08	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated, before pump P-1-F-02	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-09	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated, after pump P-1-F-02	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-10	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated, above pump P-1-F-02	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-11	Powered 2 x 2-way Sanitary Diaphragm Valve (diverter),	KSB	Type SISTO-CM2XX DN6 with butt weld ends:

SDV-1-F-12	solenoid actuated, above filter LF-1-F-01		Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-13	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated above membrane	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-14	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated above filter GF-1-F-01	KSB	Type SISTO-CAS DN20 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-15 SDV-1-F-16 SDV-1-F-17	Powered 3 x 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	KSB	Type SISTO-CM3XX DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-18	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated on top of permeate tank	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-19	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated on top of permeate tank	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-20	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated above sprayball	KSB	Type SISTO-C DN10 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-F-21 SDV-1-F-22 SDV-1-F-23	Powered 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated below permeate tank	KSB	Type SISTO-CM3XX DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
HDV-1-F-01	Manual 2-way Sanitary Diaphragm Valve, right side of permeate tank	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished;

			Diaphragm material: TFM/EPDM 2-pieces; Hand wheel PA6GF30
HDV-1-F-02	Manual 2-way Sanitary Diaphragm Valve below sample collection vessel	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; Hand wheel PA6GF30
HDV-1-F-03	Manual Sanitary Diaphragm Valve, zero dead leg, potential connection to vessel with sterile water for filter flushing	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; Hand wheel PA6GF30
HDSV-1-F-01	Manual Sanitary Diaphragm Sample Valve, in permeate line below filter LF-1-F-02	ROPLAN+MILLIPORE	Sterivalve type BOV, diaphragm Silicone; NovAseptic Connector in-line
HDSV-1-F-02	Manual Sanitary Diaphragm Sample Valve, at permeate tank	ROPLAN+MILLIPORE	Sterivalve type BOV, diaphragm Silicone; NovAseptic Connector in-line
HX-1-F-01	Cooler for effluent tank	Huber	Unichiller UC017Tw (1.7 kW at 15°C), equipped with controller “Professional”

5.2.1.5. *CIP (Cleaning-in-Place)*

The bioreactor and recirculation loop can be cleaned by filling the reactor with cleaning agents and starting the recirculation loop (Figure 12 in paragraph 5.2.3).

The cleaning will be semi-automated.

Cleaning in place is foreseen for the filtration unit and permeate line including the permeate tank. Prior to cleaning, the membrane lumen will be emptied from mixed liquor and permeate can be recovered in the permeate tank to the maximum extent possible, by using pressurized N_2 gas. Then, a CIP can be performed, after which the CIP’ed parts are emptied under 1.5 bar N_2 pressure, flushed with water, and again emptied under 1.5 bar N_2 pressure. After these procedures, the unit can be steam sterilized. Cleaning solutions and water will be pumped through the system from a cleaning buffer VSL2-1-CL-01 and recycled to it. CIP occurs simultaneously at both sides of the membrane, as it would otherwise require a lot of time for the solution to pass through the membranes. The CIP should be carefully defined to avoid contamination of the filtrate side.

A separate membrane module is connected to the CIP hardware in order to be able to COP (Clean Out of Place) a fouled membrane while the FU is working with a second membrane.

The different CIP hardware items are listed in Table 33.

Table 33. CIP hardware

Tag	Purpose/description	Supplier	ref./order n°
VSSL-1CL-01	Cleaning tank, 20 l		SS 316 L, inside Ra 0,8 µm
UF-1-CL-01	Membrane module	Novasep	Kerasep
LS-1-CL-01	Level switch HH	E+H	Liquiphant T FTL20H, Ra 0,8 µm, Tri-Clamp process connection
LS-1-CL-02	Level switch LL	E+H	Liquiphant T FTL20H, Ra 0,8 µm, Tri-Clamp process connection
TT-1-CL-01	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
P-1-CL-01	Magnetic drive centrifugal pump	Iwaki www.iwaki.be	Type YMD, SS 316L.
NOZ-1-CL-01	Spray head in influent buffer	Alfa Laval	Toftejorg SaniMidget Rotary Spray Head
NOZ-1-CL-02	Spray head in bioreactor	Alfa Laval	Toftejorg SaniMidget Rotary Spray Head
NOZ-1-CL-03	Spray head in permeate tank	Alfa Laval	Toftejorg SaniMicroRotary Spray Head
PV-1-CL-01	Powered 2-way Ball Valve, pneumatic actuated above NOZ-1-CL-01	Swagelok	SS 63G-Series, ¾”in., with position indication
PV-1-CL-02	Powered 2-way Ball Valve, pneumatic actuated above NOZ-1-CL-02	Swagelok	SS 63G-Series, ¾”in., with position indication
PV-1-CL-03	Powered 2-way Ball Valve, pneumatic actuated in line to NOZ-1-CL-03	Swagelok	SS 43G-Series, 8 mm, with position indication
PV-1-CL-04	Powered 2-way Ball Valve, pneumatic actuated, recycle CIP under membrane UF-1-F-01	Swagelok	SS 43G-Series, 8 mm, with position indication
PV-1-CL-05	Powered 2-way Ball Valve, pneumatic actuated, recycle CIP under membrane UF-1-F-01	Swagelok	SS 43G-Series, 8 mm, with position indication
PV-1-CL-06	Powered 2-way Ball Valve, pneumatic actuated, entry CIP on top of membrane UF-1-F-01	Swagelok	SS 43G-Series, 8 mm, with position indication
PV-1-CL-07	Powered 2-way Ball Valve, pneumatic actuated, entry CIP on top of membrane UF-1-F-01, permeate side	Swagelok	SS 43G-Series, 8 mm, with position indication
PV-1-CL-08	Powered 2-way Ball Valve, pneumatic actuated, recycle CIP from permeate tank	Swagelok	SS 43G-Series, 8 mm, with position indication

PV-1-CL-09	Powered 2-way Ball Valve, pneumatic actuated, recycle CIP from filter LF-1-F-01	Swagelok	SS 43G-Series, 8 mm, with position indication
NRV-1-CL-01	Safety Relief Valve	LESER	Type 437
HV-1-CL-01	Manual 2-way Ball Valve, supply water to cleaning tank	Swagelok	SS 63G-Series, ¾"in.
HV-1-CL-02	Manual 2-way Sanitary Ball Valve below cleaning tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm
HV-1-CL-03	Manual 2-way Sanitary Ball Valve below cleaning tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm
HV-1-CL-04	Manual 2-way Sanitary Ball Valve, recycle line over cleaning tank	Nocado, Alfa Laval	SS 316L, DN ≥ 25, Ra 0,8 µm
HV-1-CL-05	Manual 2-way Ball Valve, direct water supply to cleaning line	Swagelok	SS 63G-Series, ¾"in.
HV-1-CL-06	Manual 2-way Ball Valve to membrane unit	Swagelok	SS 43G-Series, 8 mm
HV-1-CL-07	Manual 2-way Ball Valve to membrane unit	Swagelok	SS 43G-Series, 8 mm
HV-1-CL-08	Manual 2-way Ball Valve from membrane unit	Swagelok	SS 43G-Series, 8 mm
HV-1-CL-09	Manual 2-way Ball Valve from membrane unit	Swagelok	SS 43G-Series, 8 mm

5.2.1.6. *Sterilisation*

The membrane filtration unit UF-1-F-01 achieves a strong reduction in bacterial cell number. As an extra safety, an in-line filter LF-1-F-02 is however provided in the permeate line. Similarly, an in-line filter LF-1-F-01 is placed in the permeate recycle line to the bioreactor to avoid direct contact between the bioreactor content and the permeate line. Gas supply to the permeate tank is also filtered (GF-1-F-02). The gas used for emptying filtration unit, piping, etc. from cleaning solutions or to maintain pressure after steam sterilization is also filtered (GF-1-F-01 and -02). Furthermore, provisions for sterile sampling have to be taken. To maintain sterility, SIP will be needed and will be implemented after CIP. The final design and lay-out should enable to perform SIP adequately. Adequate temperature should be reached without any damage to e.g. filters (due to potentially necessary increase of pressure). Insulation of the filtrate tank (including top and bottom) could be necessary.
The filtration unit, permeate line and permeate tank can be sterilised using steam. The SIP operation mode is illustrated in Figure 13 to Figure 15. Sterilization will be semi-automated. Valves and a reservoir VSSL-1-F-01 enable removal and refill of cooling liquid from the effluent vessel double jacket during the SIP procedure.

The different SIP hardware items are listed in Table 34. Points of attention are the following:

- Steam traps
  - Steam traps should be of the thermodynamic type
  - Steam traps should not come in contact with dirty product: first a rinse through side-valves is applied
  - A temperature measurement is connected to each steam trap
- Valves
  - All valves in the steam line should be membrane valves
  - Zero dead leg valves are required
  - Overpressure valves (e.g. effluent tank) should be of a sanitary type with bellow
  - Valves should be positioned at an angle of 23-26° for complete outflow
- Steaming
  - Steam sterilization starts from 1 point, and goes in 1 direction, in antenna-like approach
  - First step in SIP: dewatering of steam
  - All piping should have a slope of 1 cm/m to allow easy outflow of condensate
  - Cross-sterilization is provided for connection to next compartment
- Sensors should be mounted at a H < 3D to avoid dead zones

Table 34. SIP hardware

Tag	**Purpose/description**	**Supplier**	**ref./order n°**
HX-1-S-01			
TT-1-S-02	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-03	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-04	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-05	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-06	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-07	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-08	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-09	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-10	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection

TT-1-S-11	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-12	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-13	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-14	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-15	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
TT-1-S-16	Temperature transmitter	E+H	Easytemp TSM470F, Ra 0,8 µm, Tri-Clamp process connection
PV-1-S-01	Powered 2-way Ball Valve, pneumatic actuated	Swagelok	SS 63G-Series, ¾”in., with position indication
PV-1-S-02	Powered 2-way Ball Valve, pneumatic actuated	Swagelok	SS 43G-Series, 8 mm, with position indication
PV-1-S-03	Powered 2-way Ball Valve, pneumatic actuated	Swagelok	SS 63G-Series, ¾"in., with position indication
HV-1-S-01	Manual 2-way Ball Valve	Swagelok	SS 63G-Series, 3/4"in.
HV-1-S-02	Manual 2-way Ball Valve	Swagelok	SS 63G-Series, 3/4"in.
HV-1-S-03	Manual 2-way Ball Valve	Swagelok	SS 63G-Series, 3/4"in.
SDV-1-S-01	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-02	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-03	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces With position indication SK49
SDV-1-S-04	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-05	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-06	Powered 2-way Sanitary Diaphragm Valve, zero dead	KSB	Type SISTO-CAS DN6 with butt weld ends:

	leg, solenoid actuated		Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-07	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-08	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-09	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	KSB	Type SISTO-C DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-10	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-11	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SDV-1-S-12	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	KSB	Type SISTO-CAS DN6 with butt weld ends: Body material: SS 1.4435, inside Ra 0,8 µm, outside Ra 3,2 µm, polished; Diaphragm material: TFM/EPDM 2-pieces; With position indication SK49
SF-1-S-02	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-03	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-04	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-05	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-06	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-07	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS

SF-1-S-08	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-09	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-10	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-11	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-12	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-13	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-14	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS
SF-1-S-15	Balanced Pressure Thermostatic Steam Trap	Spirax Sarco	MST21, SS

5.2.2.P&ID

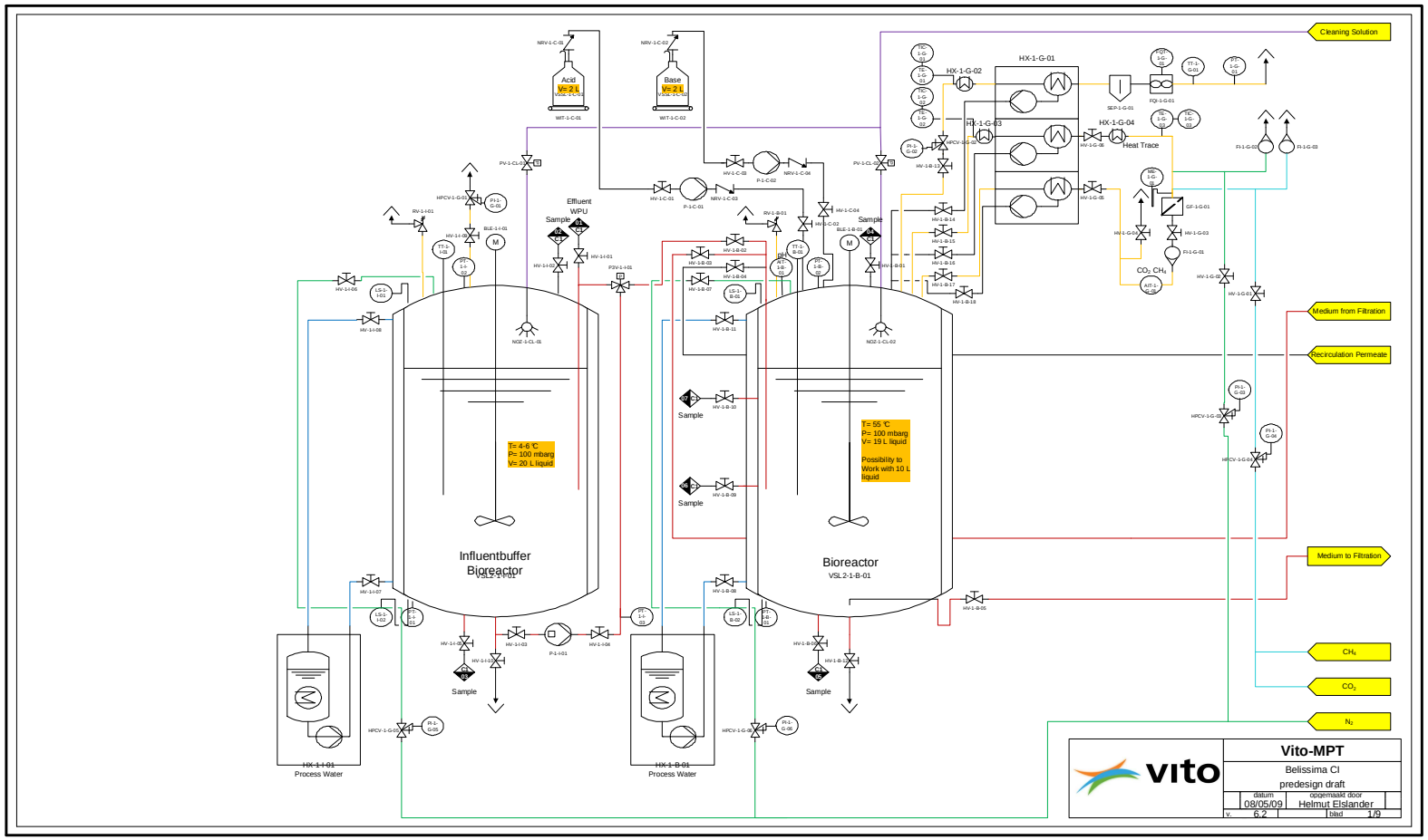


Figure 9. Overall P&ID Compartment I – influent tank and bioreactor

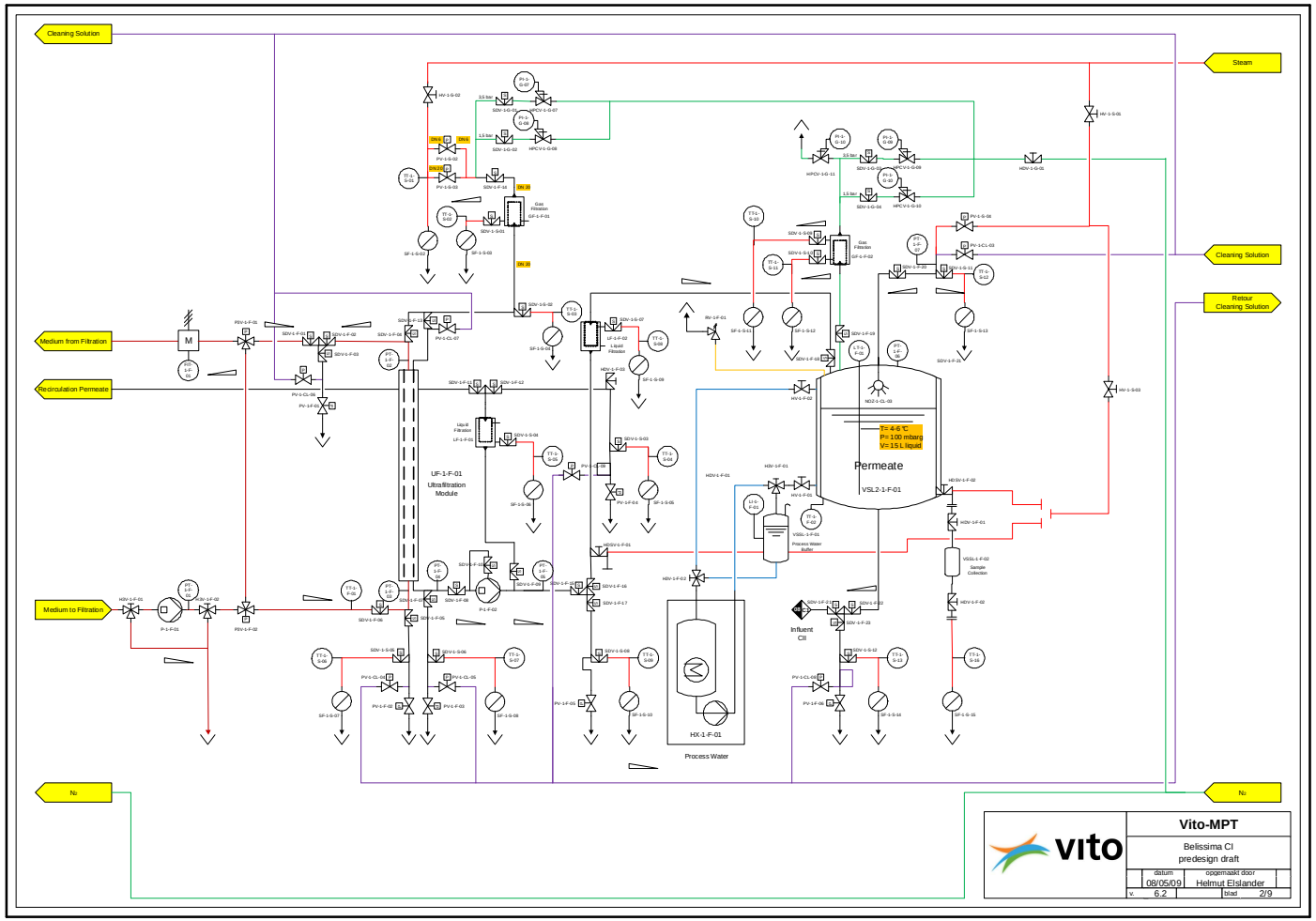


Figure 9 (continued): Filtration unit

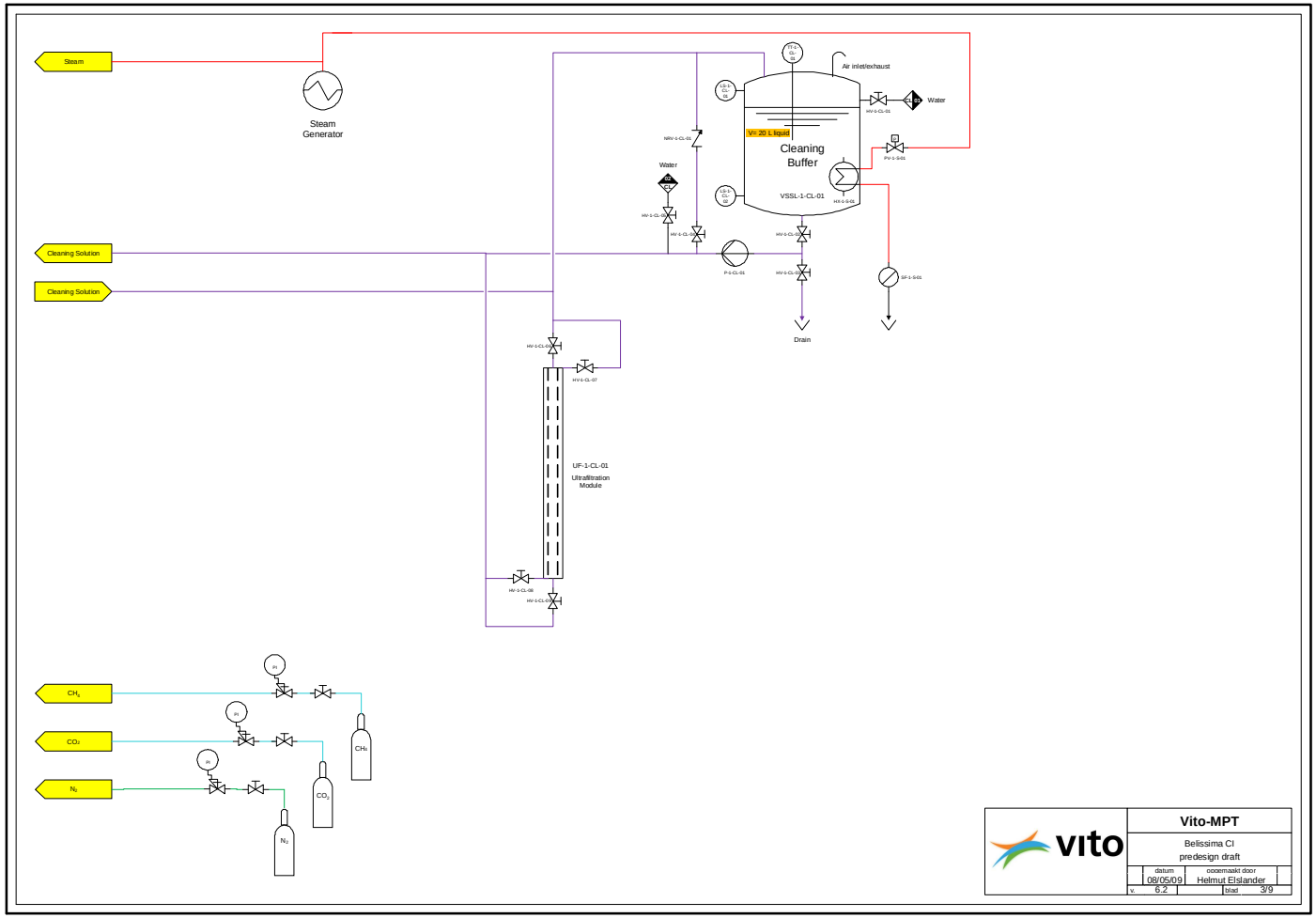


Figure 9 (continued): Utilities

5.2.3. Different operation modes

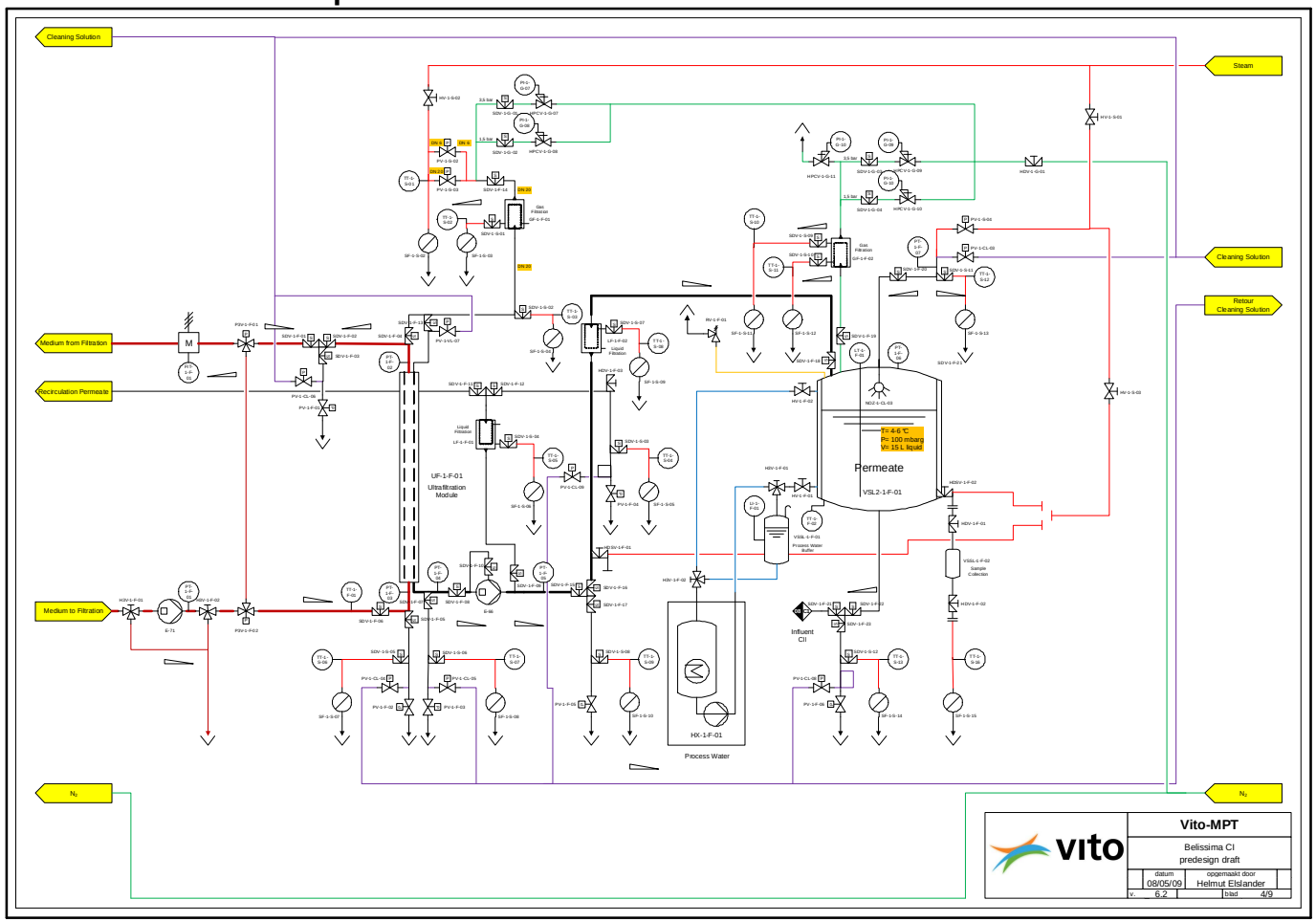


Figure 10. Filtration mode

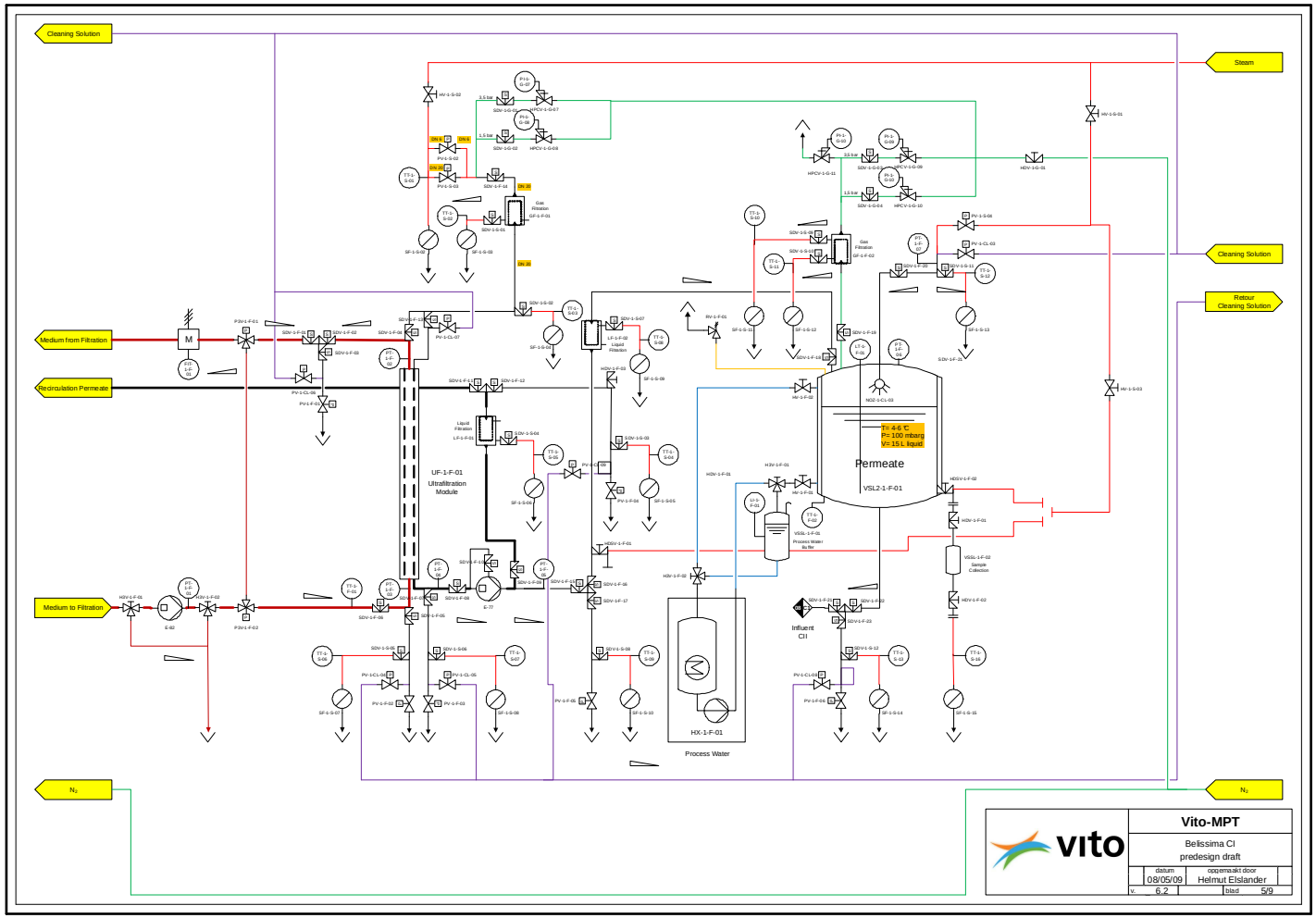


Figure 11. Recirculation permeate

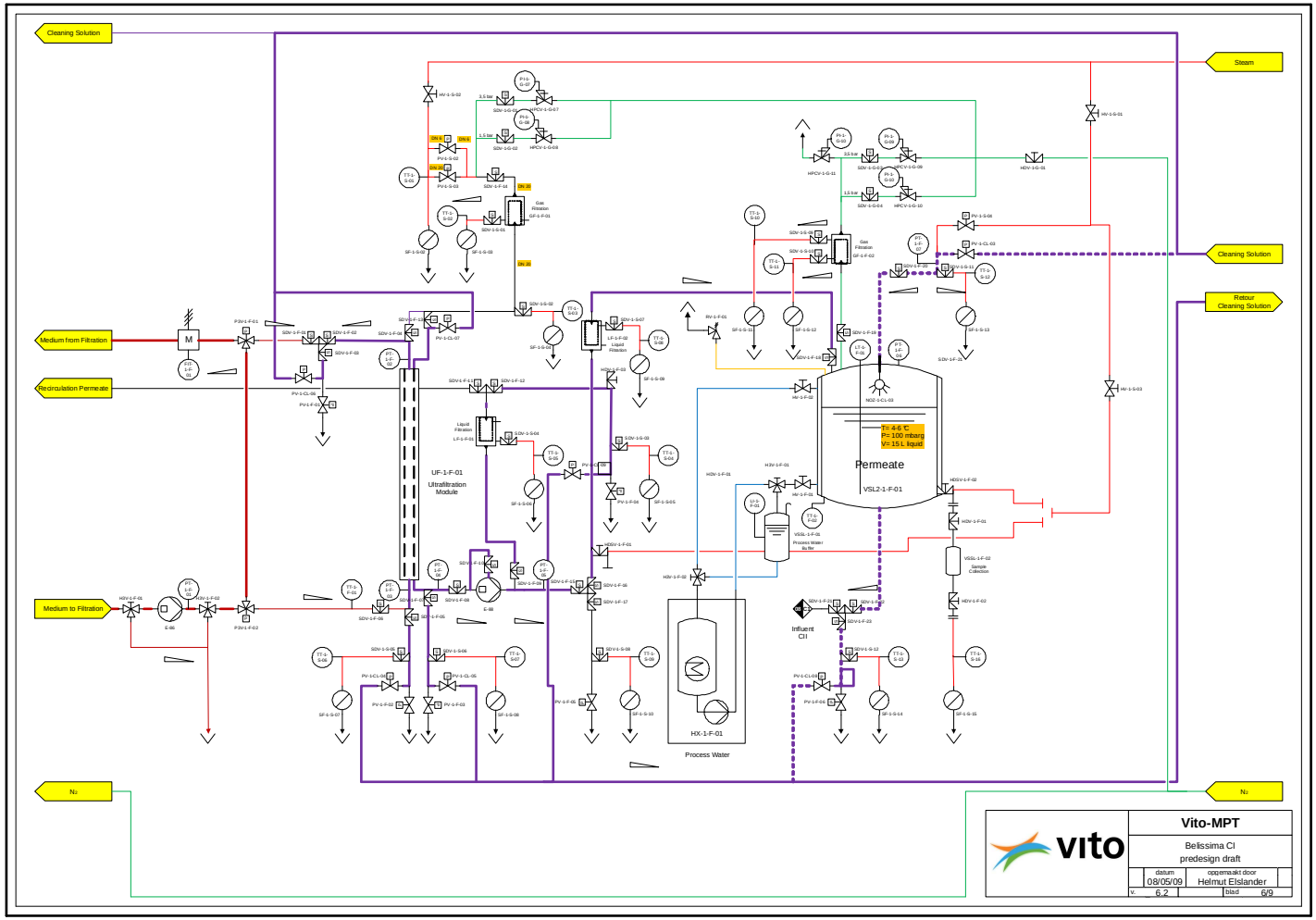


Figure 12. Cleaning in place and rinse with water (full line: part 1, dotted line: part 2)

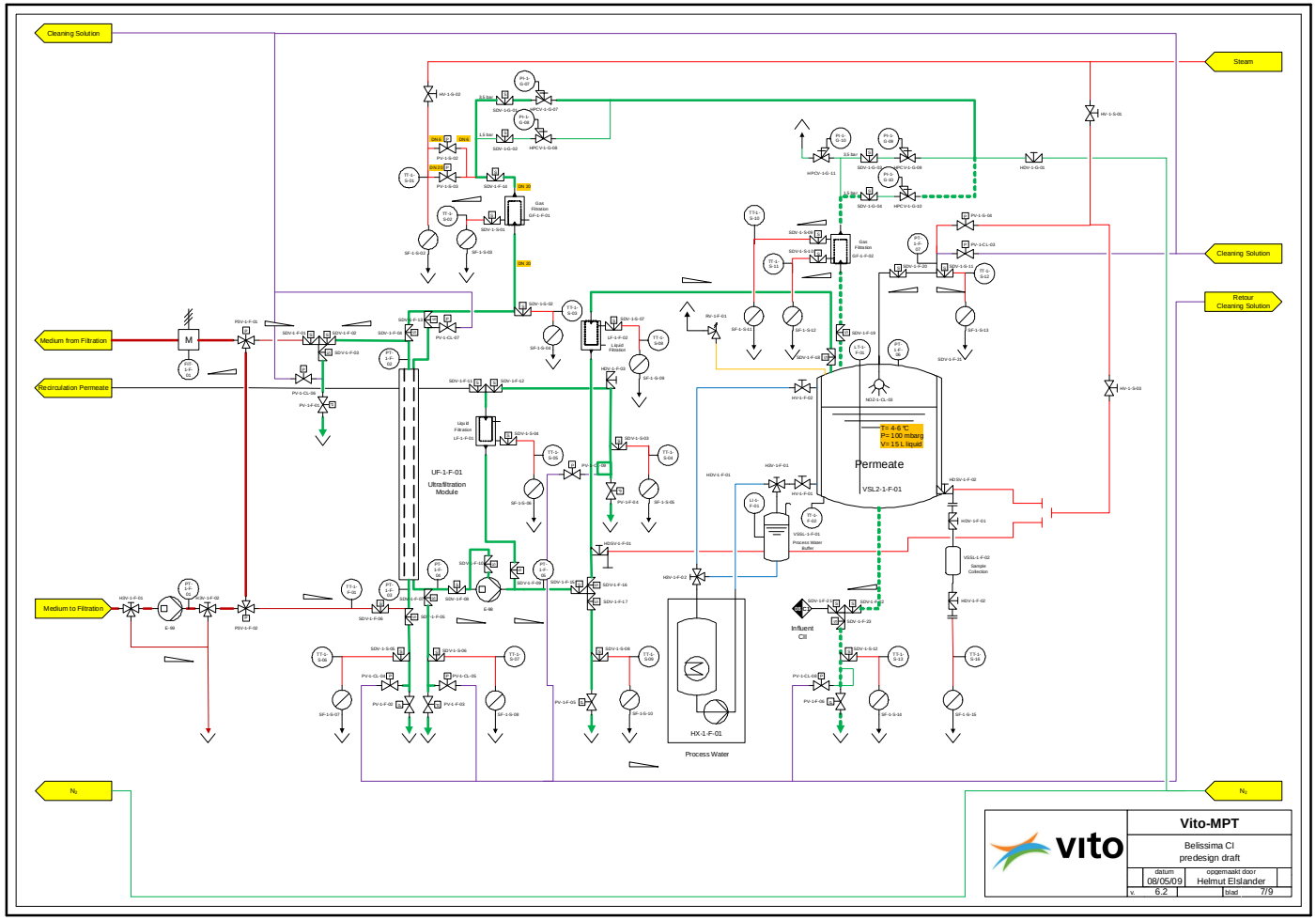


Figure 13. Purge before sterilization (full line: part 1, dotted line: part 2)

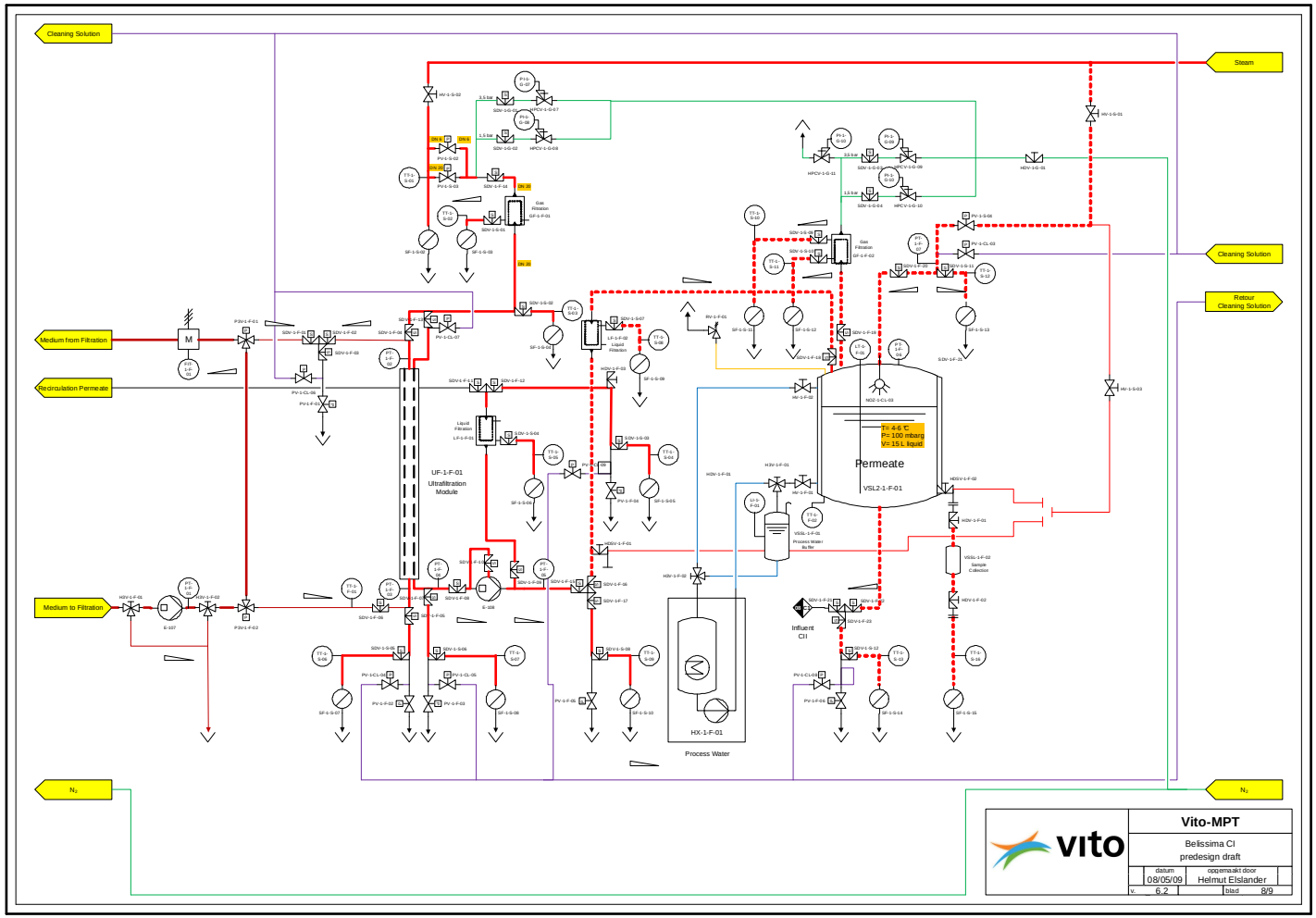


Figure 14. Steam sterilization (full line: part 1, dotted line: part 2)

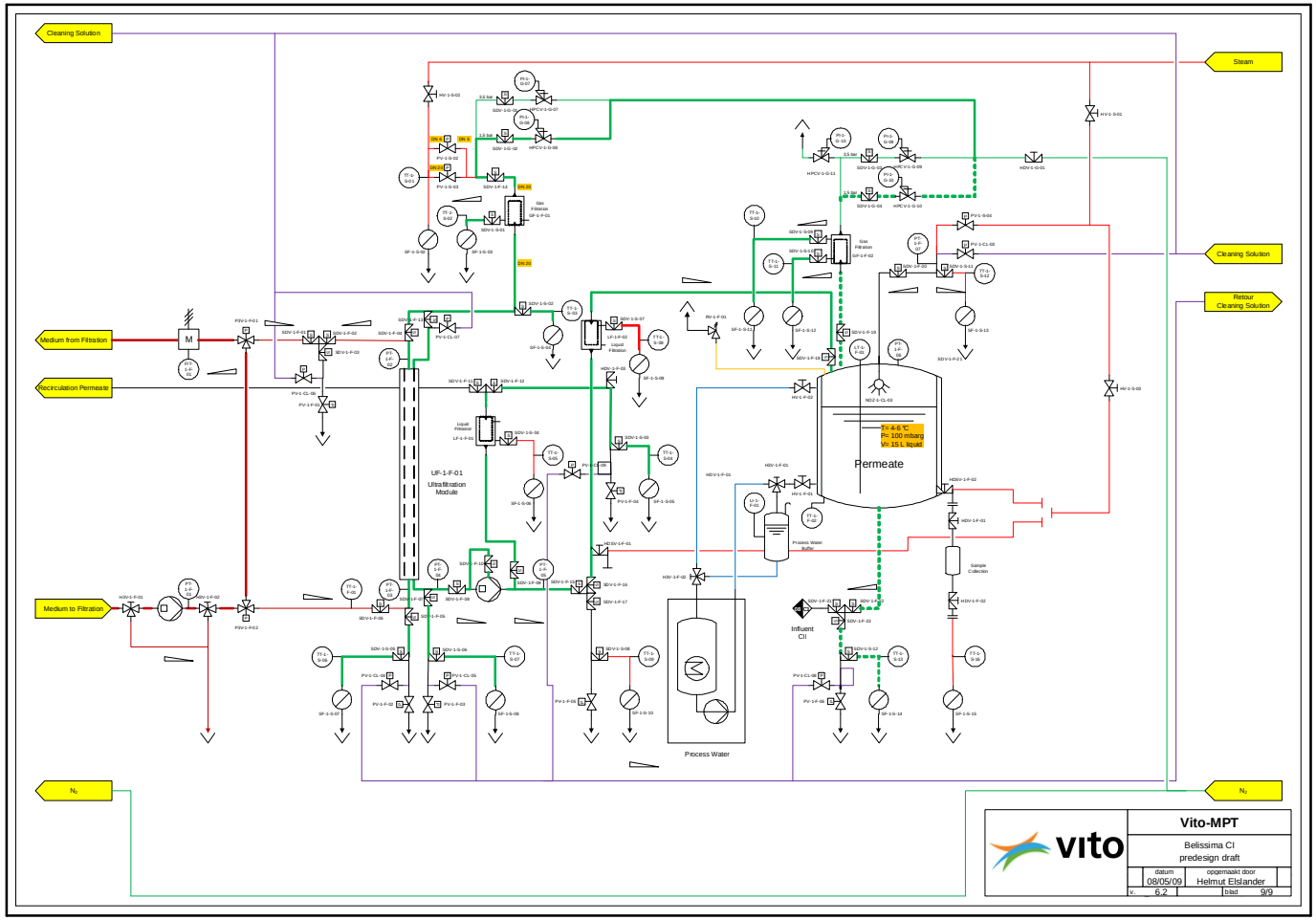


Figure 15. Dry and cool down after steam sterilization (full line: part 1, dotted line: part 2)

5.2.4.Electrotechnical data

All components of the installation that have analogue outputs, inputs, digital outputs and/or inputs are listed in Table 35.

Table 35. Electrotechnical data sheet(I/O seen from hardware)

Tag number	Description	Voltage (V)	AO	AI	DO	DI	Interface
Influent tank							
BLE-1-I-01	Stirrer with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
PT-1-I-01	Level transmitter influent tank (level measurement based on differential pressure to PT-1-I-02)	24VDC	4-20mA				
PT-1-I-02	Pressure transmitter	24VDC	4-20mA				
PT-1-I-03	Pressure transmitter after pump P-1-I-01	24VDC	4-20mA				
LS-1-I-01	Level switch HH	24VDC			x		
LS-1-I-02	Level switch LL	24VDC			x		
TT-1-I-01	Temperature transmitter	24VDC	4-20mA				
P-1-I-01	Recirculation pump influent loop with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
P3V-1-I-01	Powered 3-way Koltek Shutter valve, pneumatic actuated	24VDC			x		
HX-1-I-01	Cooler for influent tank	230VAC	4-20mA	4-20mA	x		
Bioreactor hardware							
BLE-1-B-01	Stirrer with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
PT-1-B-01	Level transmitter (level measurement based on differential pressure to PT-1-I-02)	24VDC	4-20mA				
PT-1-B-02	Pressure transmitter	24VDC	4-20mA				
LS-1-B-01	Level switch HH	24VDC			x		
LS-1-B-02	Level switch LLk	24VDC			x		

Tag number	Description	Voltage (V)	AO	AI	DO	DI	Interface
TT-1-B-01	Temperature transmitter	24VDC	4-20mA				
AIT-1-B-01	pH measurement	230VAC/24VDC	4-20mA		x		
HX-1-K-02	Heat exchanger bioreactor	230VAC	4-20mA	4-20mA	x		
WIT-1-C-01	Balance	230VAC					Ethernet
WIT-1-C-02	Balance	230VAC					Ethernet
PP1-C-01	Electromagnetic metering pump	230VAC		4-20mA	x	x	
PP1-C-02	Electromagnetic metering pump	230VAC		4-20mA	x	x	
Gas loop							
HX-1-G-01	Sample Gas Conditioner	230VAC			x		
HX-1-G-02	Heated hose with integrated temperature sensor TE-1-G-01, controller TIC-1-G-01 and exchangeable inner tube	230VAC			x		
HX-1-G-03	Heated hose with integrated temperature sensor TE-1-G-02, controller TIC-1-G-02 and exchangeable inner tube	230VAC			x		
HX-1-G-04	Heated hose with integrated temperature sensor TE-1-G-03, controller TIC-1-G-03 and exchangeable inner tube	230VAC			x		
FQI-1-G-01	Gas Counter (see FQT-1-G-01)	230VAC					
FQT-1-G-01	Pulse transmitter Gas Counter	230VAC	4-20mA				
PT-1-G-01	Pressure transmitter	230VAC	4-20mA				
TT-1-G-01	Temperature transmitter	230VAC	4-20mA				
GF-1-G-01	Aerosol, particle filter and water stop (hydrophobic filter with integrated liquid alarm sensor ME-1-G-01	230VAC			x		
AIT-1-G-01	CO_2/CH_4 IR Gas Analyzer with integrated sample gas pump, flow sensor en humidity sensor.	230VAC	4-20mA		x		
SDV-1-G-01	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-G-02	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-G-03	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
Filtration unit							

Tag number	Description	Voltage (V)	AO	AI	DO	DI	Interface
PT-1-F-01	Pressure transmitter after pump P-1-F-01	24VDC	4-20mA				
PT-1-F-02	Pressure transmitter	24VDC	4-20mA				
PT-1-F-03	Pressure transmitter	24VDC	4-20mA				
PT-1-F-04	Pressure transmitter	24VDC	4-20mA				
PT-1-F-05	Pressure transmitter	24VDC	4-20mA				
PT-1-F-06	Pressure transmitter	24VDC	4-20mA				
PT-1-F-07	Pressure transmitter	24VDC	4-20mA				
LT-1-F-01	Level transmitter	24VDC	4-20mA				
TT-1-F-01	Temperature transmitter	24VDC	4-20mA				
TT-1-F-02	Temperature transmitter	24VDC	4-20mA				
FIT-1-F-01	Flow transmitter	230VAC	4-20mA		x		
P-1-F-01	Retentate pump with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
P-1-F-02	Permeate pump with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
P3V-1-F-01	Powered 3-way Koltek Shutter valve, pneumatic actuated	24VDC			x		
P3V-1-F-02	Powered 3-way Koltek Shutter valve, pneumatic actuated	24VDC			x		
PV-1-F-01	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-02	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-03	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-04	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-05	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-06	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
SDV-1-F-01 SDV-1-F-02 SDV-1-F-03	Powered 3 x 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	24VDC			x		
SDV-1-F-04	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		

Tag number	Description	Voltage (V)	AO	AI	DO	DI	Interface
SDV-1-F-05	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-06	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-07	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-08	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-09	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-10	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-11 SDV-1-F-12	Powered 2 x 2-way Sanitary Diaphragm Valve (diverter), solenoid actuated	24VDC			x		
SDV-1-F-13	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-14	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-15 SDV-1-F-16 SDV-1-F-17	Powered 3 x 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	24VDC			x		
SDV-1-F-18	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-19	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-20	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-21	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-22 SDV-1-F-23 SDV-1-F-24	Powered 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	24VDC			x		
HX-1-K-03	Cooler for effluent tank	230VAC	4-20mA	4-20mA	x		
CIP							
LS-1-CL-01	Level switch HH	24VDC			x		
LS-1-CL-02	Level switch LLk	24VDC			x		

Tag number	Description	Voltage (V)	AO	AI	DO	DI	Interface
TT-1-CL-01	Temperature transmitter	24VDC	4-20mA				
P-1-CL-01	Magnetic drive centrifugal pump	230-400VAC/3Ph					
PV-1-CL-01	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-02	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-03	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-04	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-05	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-06	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-07	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-08	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-09	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
SIP							
TT-1-S-11	Temperature transmitter	24VDC	4-20mA				
TT-1-S-12	Temperature transmitter	24VDC	4-20mA				
TT-1-S-13	Temperature transmitter	24VDC	4-20mA				
TT-1-S-14	Temperature transmitter	24VDC	4-20mA				
TT-1-S-15	Temperature transmitter	24VDC	4-20mA				
TT-1-S-16	Temperature transmitter	24VDC	4-20mA				
PV-1-S-01	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-S-02	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-S-03	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
SDV-1-S-01	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-02	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-03	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		

Tag number	Description	Voltage (V)	AO	AI	DO	DI	Interface
SDV-1-S-04	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-05	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-06	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-07	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-08	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-09	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-10	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-11	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-12	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		

5.2.5.Process description

Start-up - Influent tank

- Prerequisite: The influent tank as well as the bioreactor must be clean and empty.
- First of all the cooling must be started in order to start cooling down the influent tank around 4 to 6 °C, by activation of HX-1-I-01. This is then circulated through the double jacket of the influent tank by the integrated pump of the cooler.
- Then the influent tank can be fed with fresh influent through the valve HV-1-I-01. The gas phase is being flushed with N_2 while HV-1-I-06 is open. It is left open for some time after all influent is fed to replace the gas phase with N_2. HPCV-1-G-05 can be set to a higher set point to increase the flow rate. One must be sure to put them back to their original settings before closing HV-1-I-06.
- The influent volume can be read on-line (measured from difference PT-1-I-01 and -02) and the user should stop filling when the maximum volume is reached in the tank.
- Then the blender BLE-1-I-01 is started in order to mix the influent. Pump P-1-I-01 is started. Once the piping connected to this pump is filled with influent P-1-I-01 should keep on working to prevent sedimentation of particles to the lower part of the piping.
- The feeding function to the bioreactor can then be started. At regular intervals of time (using a timer programmed in the PLC), valve P3V-1-I-01 is switched in the direction of the bioreactor, allowing a semi-continuous feeding mode. The settings are determined depending on the desired influent flow rate.

Start-up - Bioreactor

- Prerequisite: The bioreactor is cleaned and empty.
- First the bioreactor must be inoculated. The bioreactor must be opened (lid) and manually fed with the available volume of inoculum. A minimum volume of 10% to 20% of the desired final volume is recommended to reduce the period to start up the reactor. Inoculation should take place under nitrogen flush.
- Then the lid must be closed.
- The reactor must work in anaerobic conditions, so N_2 must be flushed to eliminate oxygen. This is done by opening manually completely pressure regulator HPCV-1-G-06 for about 2 minutes.
- Afterwards valve HPCV-1-G-06 is manually tuned to keep an overpressure in the bioreactor (PT-1-B-02, read on HMI) of about 100 mbar.
- The blender BLE-1-B-01 can then be started to homogenize the content of the bioreactor.
- Then the temperature control must be started up by activating HX-1-B-01, which will ensure a temperature of 55°C in the bioreactor. The heater is warming up water contained in a small water bath, which is then circulated through the double jacket of the bioreactor by an integrated pump.

- At that stage, the pH control can be started up. When the pH on-line measured by AIT-1-B-01 is exceeding its allowed working range (5 – 5.5), acid or base is automatically added using pumps P-1-C-01 and P-1-C-02.

Start-up - Gas loop

- Prerequisite: The bioreactor is in operation.
- HX-1-G-01 is a gas cooler with three lines and integrated pumps combined in the same instrument. When activated, the flow of gas passing through the cooler is cooled down to 4 – 6°C and the condensate is recycled to the bioreactor.
- The Gas Loop has a passive operation: when the pressure increases inside the bioreactor due to gas production above the set point (around 100 mbar), the pressure regulator HPCV-1-G-02 is opened and allows the produced gas to leave the bioreactor.
- The volume of gas leaving the bioreactor and thus - during nominal operation, when only gas production is responsible for pressure increase in the bioreactor - of gas produced is measured in the Milligas counter FQI-1-G-01. The pressure PT-1-G-01 and temperature TT-1-G-01 are measured and recorded by the PLC. The quantity of gas can be calculated from the number of switches of filled measurement chambers in the cell and corrected for pressure and temperature.
- The analyser loop can be started after HX-1-G-01 has been working for at least half an hour. The analyser may have to be calibrated before the analyser loop is started. This is done according to the procedure provided by the manufacturer, which implies that among others a flow of N_2 and of a dedicated mixture of CO_2 and CH_4 has to be sent through the analyser. N_2 is sent through the analyser and then to the exhaust by manually opening HV-1-G-02 and HV-1-G-04 and closing HV-1-G-05 and -06. The flow rate (can be read from FI-1-G-01) is manually adjusted by the needle valve of FI-1-G-02. The pressure is adjusted through pressure control valve HPCV-1-G-03. The CO_2/CH_4 mixture is sent through the analyser by opening HV-1-G-01 and -04 and closing HV-1-G-05 and -06. Its flow is regulated by the needle valve of FI-1-G-03 and its pressure by HPCV-1-G-04 as well.
- The analyser loop is started up by a command to the analyser AIT-1-G-01 that has an integrated pump and flow control. By manually opening HV-1-G-05 and -06, on-line gas analysis can be started.

Start-up - Filtration unit

- Prerequisite: The bioreactor is in operation and contains enough liquid in order to start the FU
- Valves in the FU are switched into their positions for Filtration Mode.
- P-1-F-01 is started and its rotational speed is adjusted to get the nominal flow rate in the retentate loop.
- P-1-F-02 is started with a rotational speed adapted to the desired filtrate production.
- The FU is now working in nominal operation.

Start-up in bypass mode - Filtration unit

- Prerequisite: The bioreactor is in operation and contains enough liquid in order to start the FU
- Valves P3V-1-F-01 and -02 in the FU are switched into their positions for Bypass Mode.
- P-1-F-01 is started and its rotational speed is adjusted to get the nominal flow rate in the retentate loop.
- The FU is now working in Bypass mode.

Nominal operation - influent tank

- Temperature: The influent tank is maintained at a temperature between 4 and 6°C using cooling system HX-1-I-01. The water is cooled down in the cooler, from which it is then circulated through the double jacket of the influent tank. The temperature measured in the influent tank by TT-1-I-01 is used to control the cooler (cooling set point or on/off action on cooler).
- Mixing: The influent is kept homogenous by a continuous mixing of BLE-1-I-01 and an external loop of continuous circulation activated by the pump P-1-I-01.
- Level: The influent volume is measured on line from the pressure difference between PT-1-I-01 and -02. An additional safety is foreseen with a level switch LS-1-I-01 which gives an alarm if the level is too high.
- Pressure: The pressure inside the influent tank is measured on line by PT-1-I-02. It is mechanically controlled by two manual pressure regulators: HPCV-1-G-01 and HPCV-1-G-05. A set point with a small overpressure of about 100 mbar can be given. It has the advantage to prevent underpressure as well passive inlet of oxygen. When the pressure is decreasing (e.g. while feeding the bioreactor or taking a sample), nitrogen is automatically added through HPCV-1-G-05. When the pressure is increasing (e.g. while filling the influent tank), gas can escape to the outside through HPCV-1-G-01. The tank is protected for overpressure by an overpressure relief valve RV-1-I-01.
- Filling influent tank: The influent tank can be fed regularly (for instance once a week) with fresh influent through the valve HV-1-I-01. The gas phase is being flushed with N_2 while HV-1-I-01 is open. It is left open for some time after all influent is fed to replace the gas phase with N_2. HPCV-1-G-05 can be set to a higher set point to increase the flow rate. One must be sure to put them back to their original settings before closing HV-1-I-01.
- Feeding bioreactor: At regular intervals of time (using a timer programmed in the PLC), valve P3V-1-I-01 is switched in the direction of the bioreactor, allowing a semi-continuous feeding mode by pump P-1-I-01. The settings are determined depending on the desired influent flow rate.
- Sampling, draining of influent tank: Liquid samples from the influent tank can be taken from manual valve HV-1-I-05 under the tank. Gas samples can be taken from manual valve HV-1-I-02 on top. The tank can be drained by opening PV-1-I-01.

Nominal operation - bioreactor

- Temperature: The bioreactor is maintained at a temperature of 55°C by using heat exchanger HX-1-B-01. The water is warmed up and then circulated

through the double jacket of the bioreactor. The temperatures measured in the bioreactor by TT-1-B-01 and in the heat exchanger are used to control the heating (on/off action of the heating, while the water is always circulated through the double jacket). A low level switch is integrated in the heat exchanger and gives an alarm when the level of water is getting too low due to evaporation.

- Mixing: The reactor content is kept homogenous by a continuous mixing of BLE-1-B-01.
- Level: The bioreactor volume is measured on line by a differential pressure measurement between PT-1-B-01 and -02. An additional safety is foreseen with a level switch LS-1-B-01 which gives an alarm if the level is too high and stops the influent feeding by switching valve P3V-1-I-01. The level is increasing with influent feeding and decreasing with the production of filtrate and drain.
- Pressure: The pressure inside the bioreactor is measured on line by PT-1-B-01 and PT-1-B-02. It is mechanically fixed by two manual pressure regulators: HPCV-1-G-02 and HPCV-1-G-06. A set point with a small overpressure of about 100 mbar can be given. It has the advantage to prevent underpressure and to prevent passive inlet of oxygen. When the pressure is decreasing (e.g. while draining or taking a sample), nitrogen is automatically added through HPCV-1-G-06. When the pressure is increasing (e.g. while feeding influent), gas can escape through the Gas Loop (see operation below). When the pressure is increasing brutally far above its set point (accidental), the gas can escape to the outside through RV-1-B-01, preventing risks of overpressure in the bioreactor.
- Feeding bioreactor: At regular intervals of time (using a timer programmed in the PLC), valve P3V-1-I-01 is switched in the direction of the bioreactor, allowing a semi-continuous feeding mode by pump P-1-I-01. The settings are determined depending on the desired influent flow rate.
- Producing filtrate: see filtration unit
- Sampling, draining of bioreactor: Liquid samples from the bioreactor can be taken at two levels from manual valve HV-1-B-09 and HV-1-B-10 on the tank side or from manual valve HV-1-B-06 at the bottom of the tank. The reactor can be drained through SV-1-B-01. A few litres of reactor content should be drained from time to time (frequency to be determined) to compensate bacterial growth and solids accumulation and stabilize the dry matter content. Gas samples can be taken from manual valve HV-1-B-01.
- pH control: The pH must be decreased in order to inhibit methanogenesis. When the pH on-line measured by AIT-1-B-01 is exceeding its allowed working range (5 – 5.5), acid or base is automatically added using pumps P-1-C-01 and P-1-C-02. The setpoint can be slightly modified by the operators and decreased if methane is produced.

Nominal operation - gas loop

- The Gas Loop has a passive operation: when the pressure increases inside the bioreactor due to gas production above the set point (around 100 mbar), the pressure regulator HPCV-1-G-02 is opened and allows the produced gas to leave the bioreactor.

- The flow of gas passing through the cooler is cooled down to 4 – 6°C and the condensate is recycled to the bioreactor.
- The volume of the gas leaving the bioreactor and thus - during nominal operation, when only gas production is responsible for pressure increase in the bioreactor - of the gas produced is measured in the Milligas counter FQI-1-G-01. The pressure PT-1-G-01 and temperature TT-1-G-01 are measured and recorded by the PLC. The quantity of gas can be calculated from the number of switches of filled measurement chambers in the cell and corrected for pressure and temperature. To avoid backflow of filling liquid from the chambers to the bioreactor, a separator is included.
- The analyser needs periodical calibration with N_2 and a dedicated mixture of CO_2 and CH_4. N_2 is sent through the analyser by opening valves HV-1-G-02 and -4 and closing valves HV-1-G-05 and -06. The flow rate (can be read from FI-1-G-01) is manually adjusted by the needle valve of FI-1-G-02. The pressure is adjusted through pressure control valve HPCV-1-G-03. The CO_2/CH_4 mixture is sent through the analyser by opening HV-1-G-01 and -04 and closing HV-1-G-05 and -06. Its flow is regulated by the needle valve of FI-1-G-03 and its pressure by HPCV-1-G-04 as well. Afterwards the analyser loop is restarted by opening HV-1-G-05 and -06. The flow rate (read from FI-1-G-01) is automatically controlled by AIT-1-G-01.

Nominal operation - Filtration unit

- Prerequisite: The bioreactor is in operation.
- Filtration is performed through a tubular membrane, mounted into a housing that provides connections for the retentate at both sides of the membrane. Filtrate is produced by a pressure difference over the membrane which causes a flow from the inside of the membrane to the outside.
- P-1-F-02 is a magnetic coupled pump, suited for steam sterilization. The filtrate production rate is more or less proportional to its rotational speed. This rotational speed is used to set the filtrate production rate and the equivalent flux through the membrane. As long as the membrane is not clogged the differential pressure over the membrane automatically adapts.
- P-1-F-01 pumps the reactor content to the membrane. The retentate is recycled through the membrane in order to
    - Refresh the contents of the retentate side
    - Create a certain shear on the surface of the membrane to delay fouling
    - Prevent sedimentation of particles in the retentate loop.
- PT-1-F-01 monitors pressure at the pressure side of P-1-F-01 and together with PT-1-B-01 allows to have an idea of the differential pressure over the pump. Alarms related to PT-1-F-01:
    - high high pressure: clogging and/or obstruction after P-1-F-01. P-1-F-01 is stopped.
    - high high differential pressure: clogging and/or obstruction before or after P-1-F-01. P-1-F-01 is stopped.
- PT-1-F-02, -03 and -04 monitor pressures related to the membrane. Alarms:
    - PT-1-F-02 low: abnormality. FU is stopped

  - PT-1-F-02 high: abnormality. FU is stopped
  - PT-1-F-03 low: abnormality. FU is stopped
  - PT-1-F-03 high: abnormality. FU is stopped
  - PT-1-F-04 low: abnormality. FU is stopped
  - PT-1-F-04 high: abnormality. FU is stopped
- PT-1-F-03 – PT-1-F-02 is the pressure drop between in- and outlet of the membrane. Alarms:
  - High: fouling of the membranes at retentate side. Time for cleaning/replacement.
  - High high: obstruction in or close to the membrane. FU is stopped.
- (PT-1-F-02+PT-1-F-03)/2 – PT-1-F-04 is the average transmembrane pressure between retentate side and filtrate side. Alarms:
  - High: fouling of the membrane. Time for cleaning.
  - High high: abnormality. FU is stopped.
- P-1-F-02 pushes the produced filtrate at its pressure side through a bacterial dead end filter LF-1-F-02 into the effluent vessel VSL2-1-F-01. The mechanical backpressure regulating HPCV-1-G-11 compensates the increasing liquid volume in this tank by releasing gas. This way a constant overpressure is maintained in VSL2-1-F-01.
- PT-1-F-05 measures pressure at the pressure side of P-1-F-02. Alarms:
  - Low: during nominal filtration mode, during a certain period of time no pressure increase is measured. This indicates a problem with P-1-F-02. FU is switched to bypass mode.
- TT-1-F-02 measures temperature in the effluent vessel. This temperature should be between 4°C and 6°C. Alarms:
  - Low: below 1°C effluent is about to freeze. Cooling is deactivated.
  - High: above 6°C.

Nominal operation - Harvesting

LT-1-F-01 is a continuous liquid level measurement based on microwaves that allows the PLC to calculate an approximation of the volume of liquid inside VSL2-1-F-01. At certain periods of time the effluent tank is to be harvested. Harvesting is done by closing SDV-1-F-21, opening SDV-1-F-22 and -23 and SDV-1-F-19. Pressurized N_2 pushes the liquid content out of the vessel and replaces it.

- Level Alarms for VSL2-1-F-01:
  - high high: LT-1-F-01 detects liquid. FU is switched into Bypass Mode.
  - High: Volume estimation by means of LT-1-F-01 indicates that it is time to harvest in the near future.
  - Low: after harvesting and a certain time of filtrate production, no filtrate is detected in the vessel. FU is switched into bypass mode.

Nominal operation – Cleaning Filtration unit (see Figure 12***)***

The retentate side of the membrane, including a part of the retentate piping and the filtrate side of the FU, including VSL2-1-F-01 can be cleaned and rinsed using cleaning solution (prepared or filled in cleaning vessel VSSL-1CL-01) and centrifugal pump P-1-CL-01.

- The FU must be stopped and can work in bypass mode during CIP. Pump P-1-F-02 is inactivated and bypass opened to allow sufficiently high flow of cleaning agent or water through the pump. Remaining permeate in the permeate line is recovered in the permeate tank VSL2-1-F-01 by pushing sterile N_2 via SDV-1-F-14 and SDV-1-F-13 at the permeate side of the membrane through the permeate line and dead-end filter LF-1-F-02. The permeate tank is then emptied using the harvesting procedure.
- Filters LF-1-F-01 and -02 are removed from their housing and filter housings are closed again.
- To avoid that the dirty residual at the retentate side of the membrane gets in the cleaning solution, this is first rinsed with water and the rinsing water removed to the drain.
- Chemical cleaning is performed from two different sides. On the one hand, the contents of VSSL-1CL-01 is recycled through the retentate and permeate side of the membrane, the permeate recycle line and the permeate piping (till the permeate tank). The solution is recycled via PV-1-CL-04, -05, -07 and -09. Cleaning agent will help to dissolve and remove particles. It is currently advised to keep the recycling running for more than half an hour. On the other hand, the cleaning solution is sent through the sprayball NOZ-1-CL-01 for proper cleaning of the permeate tank, and recycled through PV-1-CL-08. Afterwards, all pipings and tanks are emptied. Then, thorough rinsing with water (from VSSL-1CL-01) is necessary to remove the polluted cleaning agent from membrane, piping and tanks. This is to be repeated until the contents leaving the cleaning vessel contains no particles and has the same pH and conductivity as the water that was added to the vessel.
- Afterwards; new filters are to be mounted into the filter housing.
- Bypass over pump P-1-F-02 is to be closed if no sterilisation is scheduled afterwards.

Nominal operation – Cleaning a membrane separately

A separate membrane module is connected to the CIP hardware in order to be able to COP (Clean Out of Place) a fouled membrane while the FU is working with a second membrane. The membrane can be cleaned and rinsed using cleaning solution (prepared or filled into cleaning vessel VSSL-1CL-01) and centrifugal pump P-1-CL-01. Therefore, the fouled membrane is to be mounted into membrane module UF-1-CL-01 and the following steps are executed - using water to rinse or cleaning agent - once or repeatedly. Rinsing with water should always be performed in a last routine.

- The cleaning buffer VSSL-1CL-01 is filled with water or cleaning solution.
- According to manual settings of the valves connected to the membrane module, two different actions can be performed when P-1-CL-01 is started in a later step:
    - Cleaning/rinsing of both in- and outside of the membrane: HV-1-CL-06 to -09 are opened.
    - Only membrane inside is cleaned/rinsed: HV-1-CL-06 and -09 are opened. HV-1-CL-07 and -08 are closed.
- Heating can be activated and a temperature setpoint set.
- P-1-CL-01 is activated for a certain time (for instance 15 min).

- P-1-CL-01 is deactivated.
- The cleaning buffer tank is emptied by manipulation of HV-1-CL-03.

Nominal operation – Sterilisation Filtration unit

Both sides of the membrane and the entire filtrate side of the FU can be sterilised by Steaming in place (SIP). The membrane in its housing and the filtrate piping including the effluent vessel are heated up by means of saturated steam to a certain pressure and temperature (values to be evaluated during testing) during a certain time (that is checked for the last heat trap which reaches the required temperature).

Sterilisation must be preceded by cleaning in order to have as much retentate and filtrate residue removed as possible. After rinsing with water, the cleaned materials have to be dried with a flow of nitrogen gas at 3.5 bar. The P-1-F-02 pump head must be open and new filters must have been installed. Cooling agent is to be removed from the VSL2-1-F-01 double jacket prior to heating up. This can be done by the following steps:

- Turn off HX-1-F-01.
- Open H3V-1-F-01 to collect liquid in VSSL-1-F-01.

Sterilization with steam is performed from two different parts, and for each occurs in consecutive steps:

- Part 1
    - step 1: dewatering of steam by closing SDV-1-F-14 and directing flow to steam trap SF-1-S-02
    - step 2: sending steam to gas filter GF-1-F-01 until steam trap SF-1-S-03 closes and reaches temperature.
    - step 3: sending steam to both sides of membrane UF-1-F-01 avoiding any pressure difference over the membrane or avoiding too fast pressure increases. To enable to start with a low flow of steam and to gradually increase it, a bypass on the steam line is provided in valves PV-1-S-02 and -03. Steaming is continued until steam traps SF-1-S-07 and -08 close.
    - Step 4: sending steam through permeate recycle line through filter LF-1-F-01 until steam traps SF-1-S-05 and -06 close.
    - Step 5: send steam towards crossover triverter valves SDV-1-F-15, -16 and -17, keeping SDV-1-F-16 closed, until steam trap SF-1-S-10 closes and temperature is reached. Sterilization time is then started.
- Part 2
    - step 1: dewatering of steam by closing SDV-1-F-20 and directing flow to steam trap SF-1-S-13
    - step 2: send steam to permeate tank until steam trap SF-1-S-14 closes. Triverter valve set SDV-1-F-22, -23 and -24 form a crossover point for sterilization between compartment I and II, allowing sterile transfer of medium to the next compartment.
    - step 3: send steam to filter GF-1-F-02 until steam traps SF-1-S-11 and -12 close
    - step 4: send stem in opposite direction through filter LF-1-F-02 towards crossover sterilization point with triverter valves SDV-1-F-15, -16 and -17, now keeping SDV-1-F-15 closed. Sterilization time is

started when the required temperature is reached at steam trap SF-1-S10.

For each steaming direction, the steam is replaced with sterile N2 gas at a pressure of 1.5 bar and the system is cooled down under this atmosphere. The system (especially the membrane) has to cool down before the FU can be started. Cooling agent is filled back into the VSSL-1-F-01 double jacket:

- Turn H3V-1-F-02.
- Turn H3V-1-F-01 back to its nominal position.
- Turn on HX-1-F-01 and wait until the content of VSSL-1-F-01 is pumped back into the VSL2-1-F-01 double jacket.
- Turn H3V-1-F-02 back to its nominal position.

Transition from nominal Filtration Mode to Bypass Mode – Filtration unit

- Prerequisite: The FU is in Nominal operation
- The FU is stopped
- The FU is started up in Bypass Mode.

Transition from Bypass Mode to Nominal Filtration Mode – Filtration unit

- Prerequisite: The FU is operating in Bypass mode
- The FU is stopped
- The FU is started up in Nominal Mode.

Transition from Nominal Filtration Mode to Recycle Mode – Filtration unit

- Prerequisite: The FU is running in nominal mode.
- SDV-1-F-09 is activated.
- Filtrate is now directed back to the bioreactor instead of the effluent vessel. The FU is running and filtrating in recycle mode.

Transition from Recycle Mode to Nominal Filtration Mode – Filtration unit

- Prerequisite: The FU is running in Recycle mode.
- SDV-1-F-09 is deactivated.
- Filtrate is now directed to the effluent vessel. The FU is running and filtrating in Nominal mode.

5.2.6.Control

5.2.6.1. *Interlocking list*

TAG	Condition	Action
Influent tank R-1-I-01		
PT-1-I-01, -02	L	Alarm (Warning: time to fill influent tank)
LS-1-I-01	HH	Alarm; Disable P3V-1-I-01
LS-1-I-02	LL	Alarm; Stop P-1-I-01
PT-1-I-01	L H	Alarm; Disable P3V-1-I-01 Alarm, Disable P3V-1-I-01
PT-1-I-03	H	Alarm, stop P-1-I-01
TT-1-I-01	H L	Alarm Alarm, stop P-1-I-01

Bioreactor R-1-B-01		
PT-1-B-01, -02	H L	Alarm; Disable P3V-1-I-01 Alarm (Warning)
LS-1-B-01	HH	Alarm, Disable P3V-1-I-01; reset feeding action if it was activated
LS-1-B-02	LL	Alarm, stop FU
TT-1-B-01	H L	Alarm Alarm
AIT-1-B-01 (pH)	PV for PID control loop, SP= setpoint for pH in Bioreactor H L	MV acts on P-1-C-01 if negative and on P-1-C-02 if positive Alarm, stop P-1-C-02 Alarm, stop P-1-C-01
Filtration Unit		
FIT-1-F-01	L: ratio flow/P-1-F-01 speed < low limit value H	Alarm: warning that P-1-F-01 is operating in internal circulation Alarm; Stop FU (danger for membrane)
LT-1-F-01	HH H: volume in effluent tank >= 12L L: during harvesting L: after harvesting: no liquid detection after certain time of effluent production	Alarm; Switch to Recycle mode Alarm: warning: time to harvest effluent vessel Stop harvesting Alarm: no flow of P-1-F-02; switch to Bypass mode
PT-1-F-01	HH	Alarm: clogging, P-1-F-01 is stopped
PT-1-F-02	L: < 0 barg H: > 0,4 barg	Alarm; Stop FU Alarm; Stop FU
PT-1-F-03	L: < 0 barg H: > 0,5 barg	Alarm; Stop FU Alarm; Stop FU
PT-1-F-04	L: < -0,5 barg H: > 0,5 barg	Alarm; Stop FU Alarm; Stop FU
PT-1-F-05	L: no increase after certain time of effluent production H HH HHH: >= pressure that P-1-F-02 can deliver	Alarm: no flow of P-1-F-02; Switch to Bypass mode Alarm: pressure drop over LF-1-F-02 is increasing. Time to replace or clean the filter before next sterilisation Alarm: Instant replacement of the filter is necessary Alarm; Switch to Recycle Mode.
TT-1-F-01	L: < 50°C	Alarm
TT-1-F-02	L: < 1°C H: > 6°C	Alarm Alarm

Legend:

SP: Setpoint
PV: Process value
MV: Manipulated value
HHH: High High High limit value
HH: High High limit value
H: High limit value
L: Low limit value
LL: Low Low limit value

5.2.6.2. *Control values*

The control system for the bioreactor will be done at level 0, using a PLC. The controlled parameters are described below.

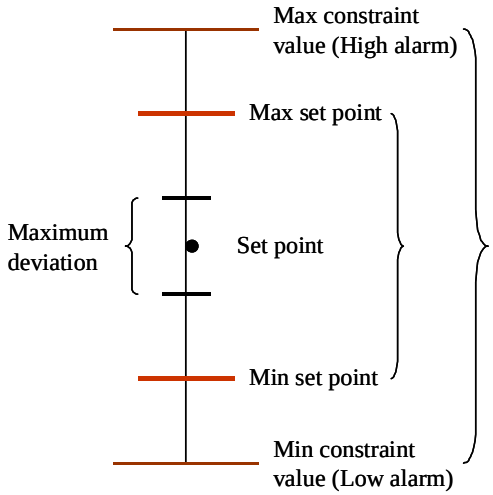


Figure 16: Definition of specifications terms

Table 36: Specifications of parameters

Specifications	T influent tank	T bioreactor	T filtrate tank
Nominal/ set point value	4 °C	55 °C	4 °C
Max. deviation	+/- 1 °C	+/- 0.5 °C	+/- 1 °C
Min. set point value	2 °C	54 °C	2 °C
Max. set point value	8 °C	56 °C	8 °C
Min. constraint value	0.5 °C	50 °C	0.5 °C
Max. constraint value	10 °C	57 °C	10 °C
Control response time			
Start-up	0.5 h	1 h	0.5 h
Nominal operation	5 min	5 min	5 min
Possible disturbances	T room Addition of influent	T room Addition of influent at 6 °C	T room Addition of filtrate at 55 °C

Specifications	pH bioreactor	Flow	Bioreactor Load
Nominal/ set point value	5.3	2 l/d	2.1 g DM/L.d
Max. deviation	+/- 0.15	+/- 10%	+/- 10 %
Min. set point value	5.15	1.8 l/d	525 mg DM/L.d
Max. set point value	5.7	2.2 l/d	4 g DM/L.d
Min. constraint value	5.1	1 l/d	263 mg DM/L.d
Max. constraint value	5.8	Not applicable	5 g DM/L.d
Control response time	5 min	0.5 h	1 d
Possible disturbances	Addition of influent (with/ without urine) Spontaneous acidification (VFA production)		Variation in waste material composition

Table 37: Specifications of pressures (differential)

Specifications	P influent tank	P bioreactor	P filtrate tank
Nominal/ set point value	100 mbar	100 mbar	100 mbar
Min. operation value	80 mbar	80 mbar	80 mbar
Max. operation value	120 mbar	120 mbar	120 mbar
Min. constraint value	5 mbar	40 mbar	5 mbar
Max. constraint value	200 mbar	200 mbar	200 mbar
Control response time	5 s	5 s	5 s
Possible disturbances	Feeding the bioreactor Filling the influent tank Opening the influent tank Nitrogen flush	Inlet influent Outlet filtrate Drain Nitrogen flush Sampling	Drain of tank Inlet filtrate

An automated detection of membrane clogging will be performed by the PLC based on pressure measurement. The automated switch to the by-pass will be stirred by the PLC in such a case.
Alarms will be programmed in case of disturbances or failures.

5.2.7.PLC IO list

Tag number	Description	Voltage (V)	AI	AO	DI	DO	Interface
	Emergency Stop	24VDC			1		
	Switch for activation of PMP-1-CL-01 during cleaning UF-1-CL-01	24VDC			1		
BLE-1-I-01	Stirrer with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
PT-1-I-01	Level transmitter influent tank (level measurement based on differential pressure to PT-1-I-02)	24VDC	4-20mA				
PT-1-I-02	Pressure transmitter	24VDC	4-20mA				
PT-1-I-03	Pressure transmitter after pump P-1-I-01	24VDC	4-20mA				
LS-1-I-01	Level switch HH	24VDC			x		
LS-1-I-02	Level switch LL	24VDC			x		
TT-1-I-01	Temperature transmitter	24VDC	4-20mA				
P-1-I-01	Recirculation pump influent loop with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
P3V-1-I-01	Powered 3-way Koltek Shutter valve, pneumatic actuated	24VDC			x		
HX-1-I-01	Cooler for influent tank	230VAC	4-20mA	4-20mA	x		
BLE-1-B-01	Stirrer with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
PT-1-B-01	Level transmitter (level measurement based on differential pressure to PT-1-I-02)	24VDC	4-20mA				
PT-1-B-02	Pressure transmitter	24VDC	4-20mA				
LS-1-B-01	Level switch HH	24VDC			x		
LS-1-B-02	Level switch LLk	24VDC			x		

TT-1-B-01	Temperature transmitter	24VDC	4-20mA				
AIT-1-B-01	pH measurement	230VAC/24VDC	4-20mA		x		
HX-1-K-02	Heat exchanger bioreactor	230VAC	4-20mA	4-20mA	x		
WIT-1-C-01	Balance	230VAC					Ethernet
WIT-1-C-02	Balance	230VAC					Ethernet
PP1-C-01	Electromagnetic metering pump	230VAC		4-20mA	x	x	
PP1-C-02	Electromagnetic metering pump	230VAC		4-20mA	x	x	
HX-1-G-01	Sample Gas Conditioner	230VAC			x		
HX-1-G-02	Heated hose with integrated temperature sensor TE-1-G-01, controller TIC-1-G-01 and exchangeable inner tube	230VAC			x		
HX-1-G-03	Heated hose with integrated temperature sensor TE-1-G-02, controller TIC-1-G-02 and exchangeable inner tube	230VAC			x		
HX-1-G-04	Heated hose with integrated temperature sensor TE-1-G-03, controller TIC-1-G-03 and exchangeable inner tube	230VAC			x		
FQI-1-G-01	Gas Counter	230VAC					
FQT-1-G-01	Pulse transmitter	230VAC	4-20mA				
PT-1-G-01	Pressure transmitter	230VAC	4-20mA				
TT-1-G-01	Temperature transmitter	230VAC	4-20mA				
GF-1-G-01	Aerosol, particle filter and water stop (hydrophobic filter with integrated liquid alarm sensor ME-1-G-01	230VAC			x		
AIT-1-G-01	CO_2/CH_4 IR Gas Analyzer with integrated sample gas pump, flow sensor en humidity sensor.	230VAC	4-20mA		x		
SDV-1-G-01	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-G-02	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-G-03	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		

PT-1-F-01	Pressure transmitter after pump P-1-F-01	24VDC	4-20mA				
PT-1-F-02	Pressure transmitter	24VDC	4-20mA				
PT-1-F-03	Pressure transmitter	24VDC	4-20mA				
PT-1-F-04	Pressure transmitter	24VDC	4-20mA				
PT-1-F-05	Pressure transmitter	24VDC	4-20mA				
PT-1-F-06	Pressure transmitter	24VDC	4-20mA				
PT-1-F-07	Pressure transmitter	24VDC	4-20mA				
LT-1-F-01	Level transmitter	24VDC	4-20mA				
TT-1-F-01	Temperature transmitter	24VDC	4-20mA				
TT-1-F-02	Temperature transmitter	24VDC	4-20mA				
FIT-1-F-01	Flow transmitter	230VAC	4-20mA		x		
P-1-F-01	Retentate pump with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
P-1-F-02	Permeate pump with frequency drive	230-400VAC/3Ph		4-20mA	x	x	
P3V-1-F-01	Powered 3-way Koltek Shutter valve, pneumatic actuated	24VDC			x		
P3V-1-F-02	Powered 3-way Koltek Shutter valve, pneumatic actuated	24VDC			x		
PV-1-F-01	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-02	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-03	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-04	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-05	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-F-06	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
SDV-1-F-01 SDV-1-F-02 SDV-1-F-03	Powered 3 x 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	24VDC			x		
SDV-1-F-04	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		

SDV-1-F-05	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-06	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-07	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-08	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-09	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-10	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-11 SDV-1-F-12	Powered 2 x 2-way Sanitary Diaphragm Valve (diverter), solenoid actuated	24VDC			x		
SDV-1-F-13	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-14	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-F-15 SDV-1-F-16 SDV-1-F-17	Powered 3 x 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	24VDC			x		
SDV-1-F-18	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-19	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-20	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-21	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-F-22 SDV-1-F-23 SDV-1-F-24	Powered 2-way Sanitary Diaphragm Valve (triverter), solenoid actuated	24VDC			x		
HX-1-K-03	Cooler for effluent tank	230VAC	4-20mA	4-20mA	x		
LS-1-CL-01	Level switch HH	24VDC			x		
LS-1-CL-02	Level switch LLk	24VDC			x		
TT-1-CL-01	Temperature transmitter	24VDC	4-20mA				

P-1-CL-01	Magnetic drive centrifugal pump	230-400VAC/3Ph			x (from contactor)		
PV-1-CL-01	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-02	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-03	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-04	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-05	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-06	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-07	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-08	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-CL-09	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
TT-1-S-11	Temperature transmitter	24VDC	4-20mA				
TT-1-S-12	Temperature transmitter	24VDC	4-20mA				
TT-1-S-13	Temperature transmitter	24VDC	4-20mA				
TT-1-S-14	Temperature transmitter	24VDC	4-20mA				
TT-1-S-15	Temperature transmitter	24VDC	4-20mA				
TT-1-S-16	Temperature transmitter	24VDC	4-20mA				
PV-1-S-01	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-S-02	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
PV-1-S-03	Powered 2-way Ball Valve, pneumatic actuated	24VDC			x		
SDV-1-S-01	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-02	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		

SDV-1-S-03	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-04	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-05	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-06	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-07	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-08	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-09	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-10	Powered 2-way Sanitary Diaphragm Valve, solenoid actuated	24VDC			x		
SDV-1-S-11	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		
SDV-1-S-12	Powered 2-way Sanitary Diaphragm Valve, zero dead leg, solenoid actuated	24VDC			x		

5.3. Overall interfaces

5.3.1.Steam production

Steam production is required for the membrane filtration unit and permeate line of compartment I. To this end, a central steam generator will be available in the dedicated facilities (6).

5.3.2.Gases

An on-line IR gas analyser will be provided for CO_2 – CH_4 analysis (see also 4.3.1). Technical gases such as N_2, CO_2, O_2, He, compressed air and natural gas will be supplied.

5.3.3.Water

In the BELISSIMA facilities both tap water, and deionized water will be available, as well as cool process water.

5.3.4.Cooling/heating equipment

Each bioreactor, influent and effluent tank will have its individual cooling and/or heating system. These are described per compartment.

5.3.5.CIP equipment

A cleaning tank, pump and associated piping, is currently included in the compartment I design but can be used in common for various compartments.

6. Available facilities

VITO will refurbish an existing building to provide dedicated laboratory facilities to house and operate the BELISSIMA loop (Figure 17). These have an overall surface area of around 250 m² including:

- a separate room to house compartment I and the Feed Preparation Unit. To avoid that spills spread over the room, provisions will be made to retain spills.
- a separate room to house compartments II to IVa
- a lab for media preparation and axenic work
- a large autoclave
- a large freezing and cooling cell
- storage capacity.

The space housing compartment I will be operated at underpressure to avoid spreading of and contamination with potentially hazardous microorganisms. Offgases will be exhausted directly to the outside of the building. The space housing the three

other axenic compartments will be operated at a slight overpressure to avoid contamination from the surrounding rooms. As the pressures in the different lab spaces vary, they can only be accessed through locks at intermediate pressure.
All labs will have common utilities, including electricity, tap water, pressurized air, gas for axenic work, air conditioning, cooled process water, etc. The floors and walls will have a finish that allows easy cleaning.

The rooms will be accessible through normal-sized double doors. Hence, all influent and effluent tanks and bioreactors will be mounted on skids with compatible dimensions.
Access will be limited to authorized personnel trained in axenic work, but there is a corridor for visitors such that visitors can see the various compartments through windows at the side walls of the labs.
The infrastructure will be compatible with Biosafety regulations. Due to the potential presence of pathogenic organisms, the labs are operated as Class II facilities. The urine and fecal material remain in the confined environment of the BELISSIMA labs. The underpressure in the space where the Feed Preparation Unit and compartment I are housed, protects the personnel against hazardous microorganisms. A H_2S sensor will be considered for biosafety reasons. Furthermore, manipulation of fecal material is limited to a minimum through the semi-automated preparation of the feed to compartment I. As previously explained in TN 80.21-23 paragraph 3.2, the design of the Feed preparation Unit is such that the risk of splashing is also minimized. The drain of compartment I will be processed as hazardous microbial waste.
Waste collection procedures are as required for Class II bacteria. Hazardous non-liquid material is stored in cardboard boxes with yellow plastic inner bags. The boxes are marked with the text ‘risicohoudend medisch afval’ (hazardous medical waste) and the UN 3291 pictogram. Hazardous liquid material and all contaminated items which can cause perforations (plastic throwaway pipets or tips) are collected in plastic closed recipients in fixed containers which are closed with a firm cover. Both types of recipients are temporarily stored (in the Class II area) in a separate box which is emptied at frequent intervals by a specialised firm. Glassware which contains contaminated material but needs to be recovered, is first decontaminated with its content by autoclaving.
The Class II area will have limited access, for authorized personnel only.

A tentative calculation on the required plant volume for operation of CI in BELISSIMA indicates that around 9 kg of red beet is needed per month, 14.3 kg of lettuce and 0.7 kg of wheat straw. The freezing cell is sufficiently large to store large quantities of the plant material. Fecal material should ideally be stored in a separate freezer.
Sampling will occur under axenic conditions. The permeate of compartment I will initially be manually transferred to compartment II which is located in another room. There will however be access through the wall between these two lab spaces as well. This will allow the direct transfer from the permeate tank of compartment I to the influent tank of compartment II. Processing of effluent of compartments II and III (by centrifugation or membrane filtration) before transfer to the next downstream compartment will occur under axenic conditions.

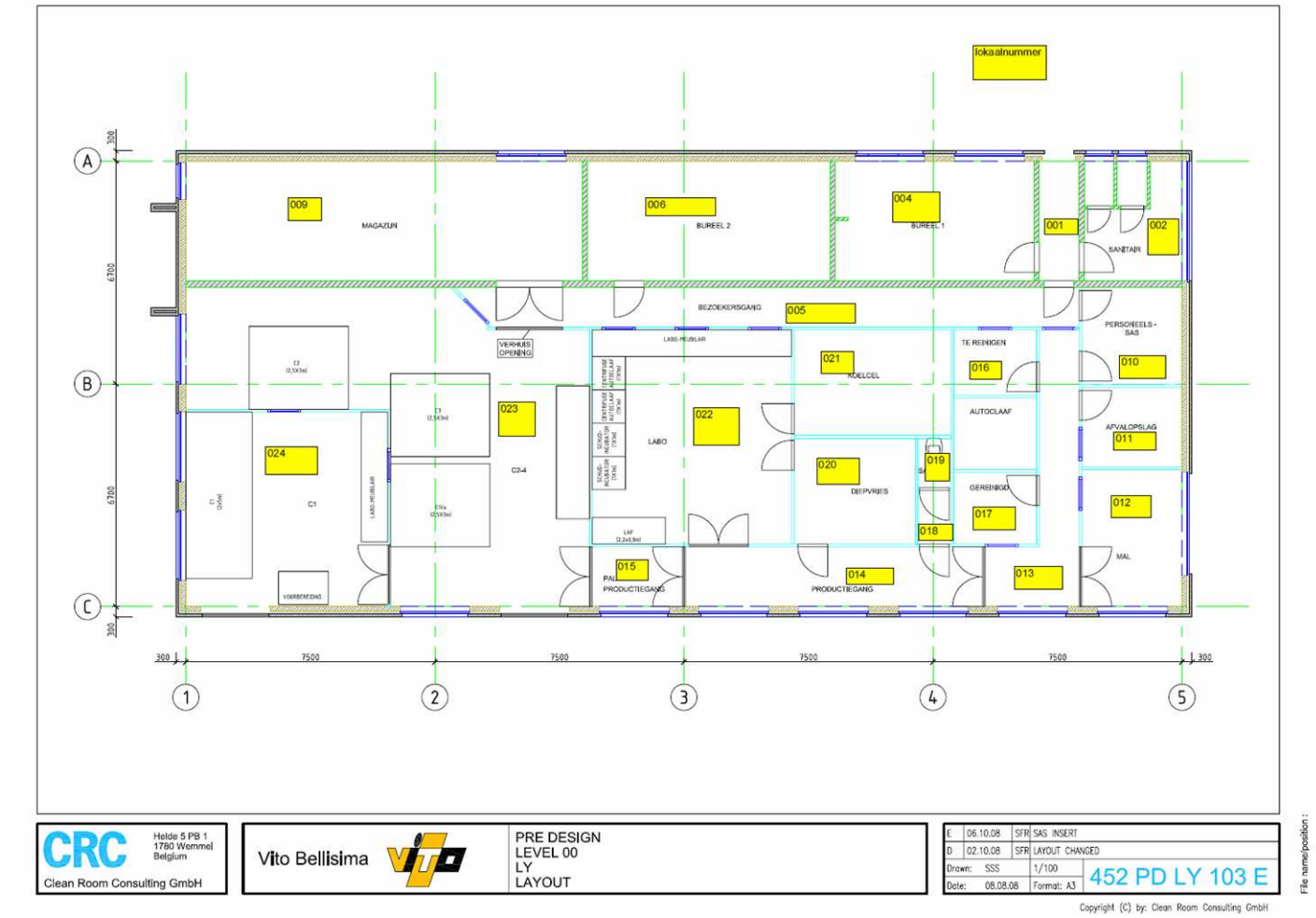


Figure 17: Schematic layout of BELISSIMA facilities.

7. Integration strategy

Compartment I is the biodegrading compartment and the point of entry for urine or for the spiking of hormones or pharmaceuticals. Because compartment I has never been coupled to the downstream compartments of MELiSSA, it will have to be tested thoroughly before coupling it to compartment II.

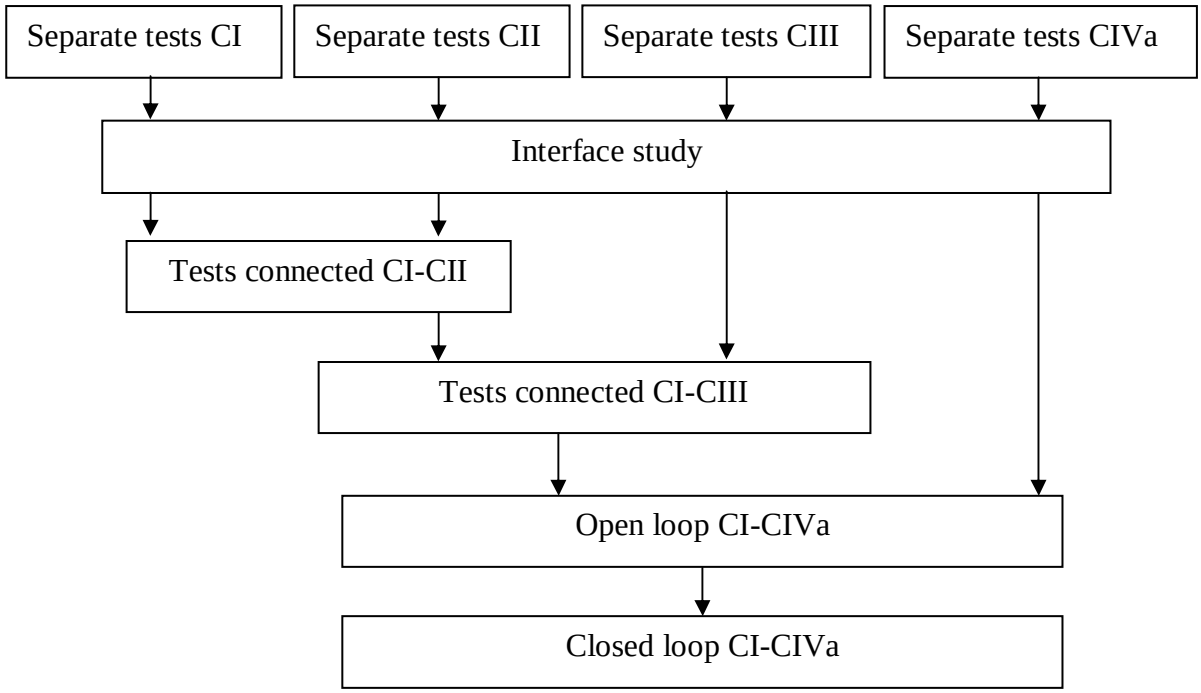


Figure 18: Integration sequence for microcompound study

Coupling of compartments will be a stepwise process. First, compartments I and II will be tested as separate units (see Figure 18). CI will in principle be available during BELISSIMA Phase 2. CII will become available in Phase 3. After axenic operation of compartment II as stand-alone unit, it will be coupled to compartment I to demonstrate that an axenic barrier can be maintained.

Each compartment will first be tested separately. This implies that the total throughput is available for microcompound analysis which poses less constraints due to sampling volumes. Therefore, each compartment will operate at its minimal working volume. For CI, this will be 12 l at a flow of 1.2 l/d. CII will operate at a working volume of around 5 l, CIII at 1 l and CIVa at 5 l. Each of them will treat flows of around 5 l/d.

Coupling of compartments will occur stepwise. First, CII will be coupled to CI. The rationale of first coupling compartment I to compartment II is based on two facts; First, this coupling is the most challenging one. Second, the real liquid output with the metabolites of the microcompounds can be fed to the next compartment, avoiding the use of synthetic media. Indeed, as CI will be the entry point for urine, and as it will be difficult to mimic the CI output detailed composition, it is our interest to study CI-CII

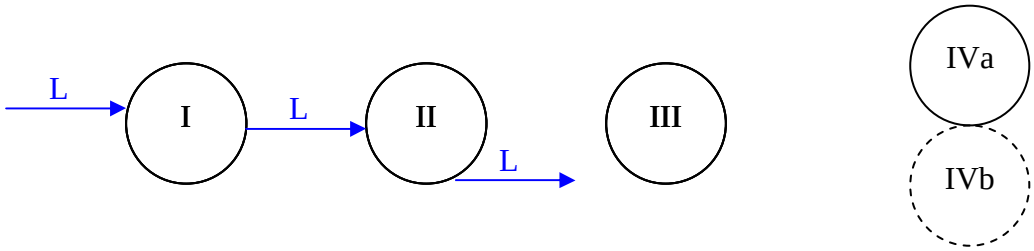
coupling as soon as possible. Then, CIII will be coupled to I and II. Finally, this connected set-up will be linked to CIVa in an open loop. The final step will consist of loop closure.
The actual time to couple CII to CI will most probably be when the effect of male hormones is investigated in CI. At this point, metabolites may occur in the effluent of CI and this specific liquid phase must be collected and transferred to CII. Upon coupling and in view of hormone analysis, sampling volumes may already become an issue and the working volume of CII may have to be doubled to 10 l to increase the liquid flow to around 10 l/d.

As specified before, the choice of the harvesting system after CII can only be evaluated once the CII biomass is available. Hence, the interphase study will have to run in parallel to the separate tests with CII. The same is true for CIVa.

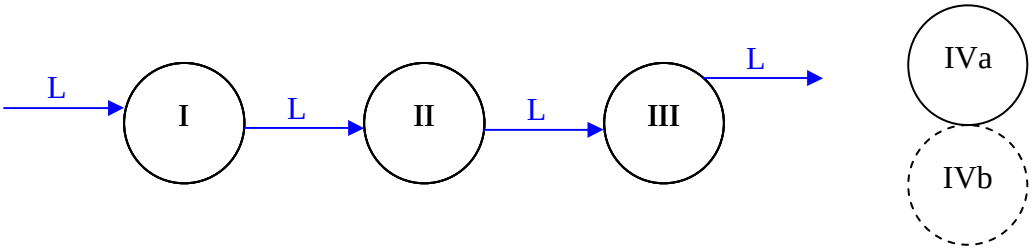
At a later stage beyond the first BELISSIMA contract, the integration of compartments III and IVa and a higher plant compartment can be considered.

On the following pages, the 6 steps envisaged for the integration strategy with respect to liquid loop closure are described. Full details on set-points, culture conditions, follow-up, etc. form part of the development of test plans, which is not the scope of this TN and will be tackled in later workpackages of the BELISSIMA contract.

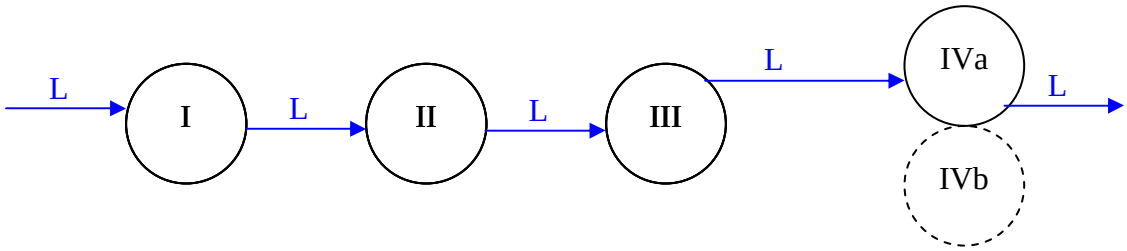
Step	1	Title	Connection CI-CII	
Inputs	Hardware	CI fully characterized and impact of male hormones tested CII at operational steady state and axenic operation demonstrated Interface liquid phase CI-CII (if any)		
	Software	Compartments connected to the supervision system		
	Knowledge	Full characterization of CI and CI liquid output		
Content	Constraint	Effect of urine addition on CI operation Effect of feed composition (different scenario investigated in BELISSIMA compared to MELiSSA) on CI operation CII liquid input composition in relation to CI liquid output composition		
	Manipulable inputs	Dilution between compartment I and II CI residence time CII light and residence time		
	Set-point	VFA concentration in input CII		
	Culture conditions	Real liquid output coming from CI		
	Follow-up	CI: liquid input composition, liquid output composition, gas composition CII: liquid output composition, biomass composition		
	Quality control	CII axeny		
Objectives/ outputs	Demonstrate that axenic barrier between CI and CII can be maintained Investigate effect/behaviour of microcompounds or their transformation products in coupled set-up CI-CII			
Remarks	Liquid phase connection aims initially at “manually “connected compartments			



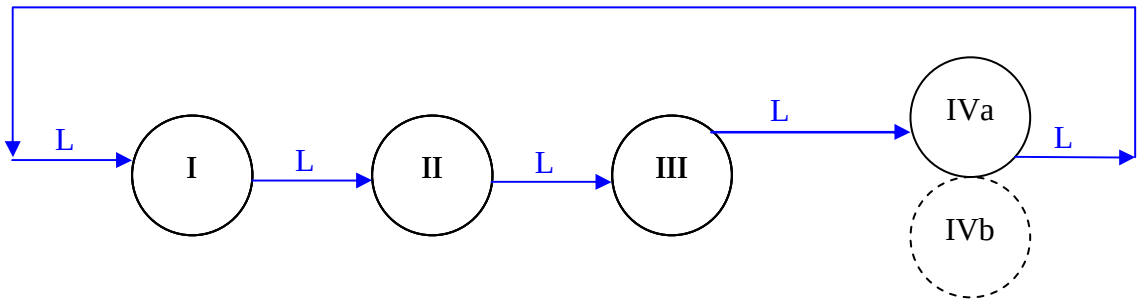
Step	2	Title	Connection CIII to CI-CII	
Inputs	Hardware	CI-CII connected as from step 1 CIII at operational steady state and axenic operation demonstrated *R. rubrum* harvesting system Additional interface to remove potential residual VFA in CII output		
	Software	Compartments connected to the supervision system		
	Knowledge	CII liquid output composition Results of step 1		
Content	Constraint	Ammonia concentrations in effluent CII due to urine addition to CI input		
	Manipulable inputs	Dilution between compartment I and II CI residence time CII light and residence time CIII residence time and oxygen input		
	Set-point	Nitrate concentration output CIII		
	Culture conditions	Real liquid outputs		
	Follow-up	CI: liquid input composition, liquid output composition, gas composition CII: liquid output composition, biomass composition CIII: liquid output composition		
	Quality control	axeny CII and CIII		
Objectives/ outputs	Demonstrate that axenic barrier between CII and CIII can be maintained Investigate effect/behaviour of microcompounds or their transformation products in coupled set-up CI-CIII			
Remarks	Liquid phase connection aims initially at “manually “connected compartments			



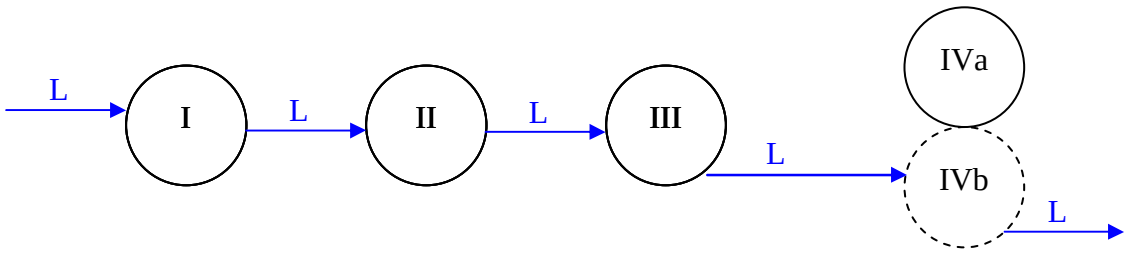
Step	3	Title	Connection CIVa to CI-CII-CIII	
Inputs	Hardware	CI-CIII connected as from step 2 CIVa at operational steady state and axenic operation demonstrated Interface between CIII and CIVa (if any)		
	Software	Compartments connected to the supervision system		
	Knowledge	CIII liquid output composition Results of step 2		
Content	Constraint	CIII dilution rate		
	Manipulable inputs	Dilution between compartment I and II CI residence time CII light and residence time CIII residence time and oxygen input CIVa light, CO_2 addition and residence time		
	Set-point	O_2 production in CIVa		
	Culture conditions	Real liquid outputs		
	Follow-up	CI: liquid input composition, liquid output composition, gas composition CII: liquid output composition, biomass composition CIII: liquid output composition CIVa: liquid output composition, biomass composition		
	Quality control	Axeny CII, CIII and CIVa		
Objectives/ outputs	Demonstrate that axenic barrier between CIII and CIVa can be maintained Investigate effect/behaviour of microcompounds or their transformation products in coupled set-up CI-CIVa – open loop			
Remarks	Liquid phase connection aims initially at “manually “connected compartments			



Step	4	Title	Closing the liquid loop CI-CII-CIII-CIVa	
Inputs	Hardware	CI-CIVa connected as from step 3 Interface between CIVa and CI		
	Software	Compartments connected to the supervision system		
	Knowledge	CIVa liquid output composition Results of step 3		
Content	Constraint	Presence of residual solutes in liquid output CIVa		
	Manipulable inputs	Dilution between compartment I and II CI residence time CII light and residence time CIII residence time and oxygen input CIVa light, CO_2 addition and residence time Composition of liquid output CIVa		
	Set-point	O_2 production in CIVa		
	Culture conditions	Real liquid outputs		
	Follow-up	CI: liquid input composition, liquid output composition, gas composition CII: liquid output composition, biomass composition CIII: liquid output composition CIVa: liquid output composition, biomass composition		
	Quality control	Axeny CII, CIII and CIVa		
Objectives/ outputs	Investigate effect/behaviour of microcompounds or their transformation products in closed liquid loop			
Remarks	Liquid phase connection aims initially at “manually “connected compartments			



Step	5	Title	Connecting CIII to CIVb	
Inputs	Hardware	CI-CIII connected as from step 2 CIVb at operational steady state Interface between CIII and CIVb (if any)		
	Software	Compartments connected to the supervision system		
	Knowledge	Results of step 4		
Content	Constraint	Composition of CIII liquid outlet		
	Manipulable inputs	Dilution between compartment I and II CI residence time CII light and residence time CIII residence time and oxygen input CIVb light, liquid composition and residence time		
	Set-point	CIVb biomass production		
	Culture conditions	Real liquid outputs		
	Follow-up	CI: liquid input composition, liquid output composition, gas composition CII: liquid output composition, biomass composition CIII: liquid output composition CIVb: composition of plants, gas phase composition		
	Quality control	Axeny CII and CIII, microbiological control CIVb		
Objectives/ outputs	Investigate effect/behaviour of microcompounds or their transformation products on higher plant compartment			
Remarks	Liquid phase connection aims initially at “manually “connected compartments Total flow is redirected from CIVa to CIVb			



Step	6	Title	Closing the liquid loop CI-CII-CIII-CIVa	
Inputs	Hardware	CI-CIVb connected as from step 5 Interface between CIVb and CI (if any)		
	Software	Compartments connected to the supervision system		
	Knowledge	Results of step 5		
Content	Constraint			
	Manipulable inputs	Dilution between compartment I and II CI residence time CII light and residence time CIII residence time and oxygen input CIVb light, liquid composition and residence time		
	Set-point	CIVb biomass production		
	Culture conditions	Real liquid outputs		
	Follow-up	CI: liquid input composition, liquid output composition, gas composition CII: liquid output composition, biomass composition CIII: liquid output composition CIVb: composition of plants, gas phase composition		
	Quality control	Axeny CII and CIII, microbiological control CIVb		
Objectives/ outputs	Investigate effect/behaviour of microcompounds or their transformation products in closed liquid loop including higher plant compartment			
Remarks	Liquid phase connection aims initially at “manually “connected compartments