TECHNICAL NOTES

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Compartment design
Associated instrumentation and control hardware
Interfaces between compartments

Prepared by/Préparé par	W. Schiettecatte, H. Elslander, J. Vereecken, N. Michel, J.-F. Cornet, H. De Wever
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Author *Auteur*	W. Schiettecatte, H. Elslander, J. Vereecken, N. Michel, J.-F. Cornet, H. De Wever	Date *Date*	24/08/2007

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G. Dussap	UBP	electronic
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1 Introduction

BELISSIMA aims to study the behaviour of microcompounds in the MELiSSA loop. In TN80.11, 80.12 and 80.14 an overview was given of all potential study items, which were then ranked according to priority. In TN80.15, an overall research plan was developed taking into account some limiting factors and critical issues related to microcompound study. In TN80.16, the corresponding requirements for the BELISSIMA loop were listed to ensure that the final loop design will have the necessary features to accommodate for relevant microcompound studies over a long period of time. In this technical note, the design of each compartment is studied and proposed.

2 First design options

2.1 Material selection

As the BELISSIMA study will focus on the behaviour of micro-compounds in the MELiSSA loop, the material selection for the reactors must be well-considered. For all four compartments the material requirements were given in TN80.16. Summarized, the requirements are:

- ✓ resistant to corrosion;
- ✓ resistant to biofilm formation;
- ✓ resistant to chemical cleaning and desinfection;
- ✓ resistant to steam sterilisation, preferable in-line;
- ✓ heat and pressure proof.

Inert material will be needed for the BELISSIMA study. The release of organic (endocrine disrupting compounds,…) and inorganic compounds (mainly metals) should be minimal. Pharmaceutical grade of material can be taken as a standard for BELISSIMA. This implies the following:

- ✓ Stainless steel with an Ra <= 0,8 µm
- ✓ Fittings and tubings with negligible leaching (teflon, C-Flex, Platinum cured, …)

For the bioreactor material and the tubing an overview is given of the different available materials.

2.1.1 Selection of tubing material (see also TN71.2)

Generally the following statements can be made:

1. Metals are non permeable, but mostly sensitive to corrosion. Special steel types and alloys show an increased resistance to corrosion. The most commonly used material is stainless steel (AISI-316). The composition as well as the polishing of stainless steel can vary.

2. Polyvinyl chloride (PVC) is lightweight, chemical and water resistant. It is easy to use and install and it is an economic solution. The disadvantage of PVC is its lower temperature resistance on the long term. However, for PN16 tubing, a life time of 25 year is guaranteed at 4 bar and 60°C.
3. The most inert material is glass. However, the limited compatibility of glass with pressure is a limiting factor in the use of glass.

2.1.2 Selection of bioreactor material (see also TN71.2)

2.1.2.1 Introduction

Three different materials can be taken into consideration for the construction of reactors: polymers, stainless steel and glass.

2.1.2.2 Glass

DURAN is a borosilicate glass. DURAN glass used in laboratories and industrial plants has excellent chemical and physical properties. The glass has the following composition:

- ✓ 81% SiO_2
- ✓ 13% B_2O_3
- ✓ 4% Na_2O/K_2O
- ✓ 2% Al_2O_3

Borosilicate glass is highly resistant to water, acids, salt solutions, organic substances and halogens such as chlorine and bromine. It has also relatively good resistance to alkaline solutions. Only hydrofluoric acid, concentrated phosphoric acid and strong alkaline solutions cause appreciable surface removal of the glass at higher temperatures. The glass can be sterilised with hot air or steam.

It is however fragile, it cannot be subjected to abrupt changes in temperature and its resistance to pressure is limited. Furthermore, it is difficult to handle and to adapt in terms of addition of connectors and fittings.

2.1.2.3 Polymers

Two types of polymers can be distinguished: amorphous and crystalline polymers.

Amorphous polymers: e.g. PVC, Acrylic have:

- ✓ a wide melting range,
- ✓ low shrinkage after molding,
- ✓ lower chemical resistance than crystalline polymers,

Polyvinyl chloride (PVC), is made up of 40% petroleum and 60% salt. The characteristics of polyvinyl chloride make it suitable for a great number of products. PVC is water resistant, resists decay quite well and is lightweight. PVC's characteristics provide many benefits:

- ✓ High strength and durability
- ✓ Easy fabrication and use

- ✓ Thermoforming
- ✓ Dimensional stability
- ✓ Easy cleaning
- ✓ Economical

Disadvantages are however the moderate temperature and chemical resistance, especially towards solvents.

- ✓ moderate heat resistance.

Crystalline polymers: e.g. Polyethylene (PE), Polypropylene (PP) have:

- ✓ a sharply-defined melting point,
- ✓ high shrinkage after molding,
- ✓ high heat resistance,
- ✓ good fatigue endurance,
- ✓ good chemical resistance.

Polymers are generally easy to glue and/or solder.

Polypropylene (PP) is a long chain polymer made from propylene monomers. Several different polymerisation methods are used to produce polypropylene. Polypropylene has excellent heat resistance and can be used in continuous processes at temperatures as high as 104 °C (Figure 1).

In general, polypropylene is not susceptible to environmental stress cracking, and it can be exposed under load in the toughest environments. Due to its high crystallinity, polypropylene has excellent moisture barrier properties and good optical properties. High crystallinity improves stiffness, but reduces impact strength.

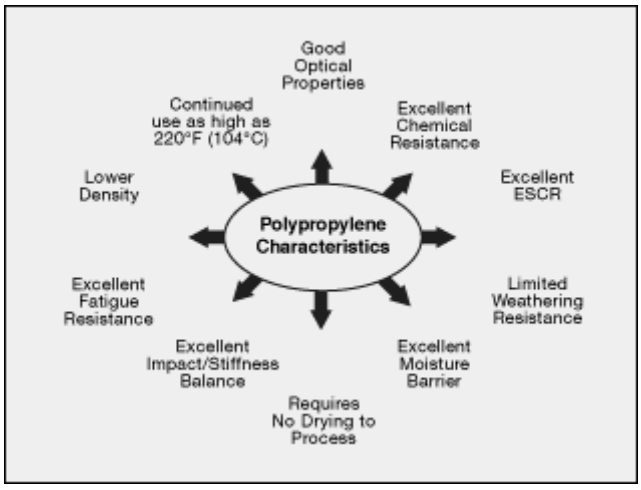

Figure 1: Characteristics of PP

Polyethylene (PE) is commercially available in 5 grades, where the higher density PEs are more crystalline in nature:

- ✓ High density (HDPE)
- ✓ Low density (LDPE)
- ✓ Linear low density (LLDPE)
- ✓ Very low density (VLDPE)
- ✓ Ultra high molecular weight (UHMWPE)

It should be noted that even the high-density PE, has only a low to moderate temperature resistance.

Chemical resistance of polymers

In Table 1, the chemical resistance is compared for PVC, PP and PE.

Table 1: Chemical resistance of 4 different polymers

	PVC	**PP**	**PE**
Resistant to	Acids, bases	Acids, bases and weak solvents	
Not resistant to	Solvents, aromatics	Oxidative acids, halogens	

Chemical disinfectants for polymers

The chemical disinfectants are generally liquid solutions or alcohols containing special anti-microbial substances and tensio-active agents. The latter, due to their capillary effect, increase the bactericidal effect. Despite the fact that pH varies slightly during the disinfection, this does not have any influence on the resistance capacity of the plastic material. However, in case a polymer is used for the reactor construction, care should be taken with the selection of the disinfectant, since certain polymers can be sensitive to them.

Temperature resistance of polymers

Table 2 shows the temperature resistance for PVC, PP and PE.

Table 2: Temperature resistance for 4 different polymers

Material	**Maximal admissible temperature (°C)**	
	Continuous	**Batch**
PVC-C (extra-chlorinated)	90	110
PVC-U (Unplastified)	60	60
PP-HT (High Temperature)	90	100
PE-HD (High Density)	60	80

2.1.2.4 Metals/Alloys

The most commonly used metals/alloys for reactors are stainless steel, carbon steel, monel, hastalloy C and Titanium. A short description of the metals/alloys is given below:

Stainless steel

Stainless steel is an alloy of iron, chromium, nickel, carbon, and other materials. The various types possess different mechanical and physical properties. These properties are, in most instances, vastly different from low carbon (mild), medium carbon and low alloys steels, with a corresponding effect on the cutting methods and procedures.

Categories of stainless steels:

- **Austenitic -** A family of alloys containing chromium and nickel (and manganese and nitrogen when nickel levels are reduced). These alloys are not hardenable by heat treatment. This group offers the highest resistance to corrosion.
- **Ferritic -** This group of alloys generally contains only chromium, with the balance mostly Fe. These alloys are somewhat less ductile than the austenitic types and again are not hardenable by heat treatment.
- **Martensitic -** The members of this family of stainless steels may be hardened and tempered just like alloy steels. Their basic building block consists of 12% Cr, 0.12% C, and balance mostly Fe.
- **Precipitation-Hardening -** These alloys generally contain Cr and less than 8% Ni, with other elements in small amounts. As the name implies, they are hardenable by heat treatment.
- **Duplex -** This is a stainless steel alloy group, or family, with two distinct microstructure phases, ferrite and austenite. The Duplex alloys have greater resistance to chloride stress corrosion cracking and higher strength than the other austenitic or ferritic grades.
- **Cast -** The cast stainless steels, in general, are similar to the equivalent wrought alloys. Most of the cast alloys are direct derivatives of one of the wrought grades. The alloy is primarily used for resistance to liquid corrosion.

The advantages and disadvantages of the different types of stainless steel are summarised in Table 3.

Table 3: Advantages and disadvantages of the different applicable types of stainless steel

Type	Examples	Advantages	Disadvantages
Ferritic	410, 430	Low cost, moderate corrosion resistance & good formability	Limited corrosion resistance, formabilty & elevated temperature strength compared to austenitics
Austenitic	304, 316, 317	Widely available, good general corrosion resistance, good cryogenic toughness. Excellent formability & weldability	Work hardening can limit formability & machinability. Limited resistance to stress corrosion cracking
Austentic	254 SMO	High resistance to pitting an crevice corrosion, very high resistance to chloride stress corrossion	Work hardening can limit formability & machinability.

If the selected metal material will be of stainless steel, the choice will be between stainless steel 316 (which is widely available), 317 or 254 SMO dependent on the need to resist corrosion (mainly by Cl^-). It should be noted that stainless steel AISI-316 is an industry standard for easy interfacing, a proven technology for sensor fittings (which are mostly in 316L/PVDF).
Polishing of stainless steel is also important to prevent corrosion and contaminations. The stainless steel surface properties depending on the polishing are presented in Table 4.

Table 4: Different polishing options for stainless steel

Nominal Grit	Ra measurement		Typical applications and techniques
	Micro-meter	Micro-inch	
80	2.5	100	Dull polish - applicable for tube, bends and tees in general food processing subject to daily cleaning in place (CIP). Normal abrasives required.
100	1.2	48	
120	1.0	40	
150	0.8	32	Satin polish - used by high end food and beverage plants, cosmetics, toiletries and with standard pharmaceutical and biotechnology applications. Easier to CIP. Sequential use of abrasives is required.
180	0.6	24	
240	0.5	20	
320	0.4	16	Bright polish - Top end pharmaceutical (water for injection), high value biotechnology materials, semi-conductors, solvents and pure gases. Abrasives plus electropolishing normally required.
500	0.05 - 0.2	4 - 8	

High-carbon steels
High-carbon steels are extremely strong but more brittle and therefore harder to form and weld. This composition however allows better responses to heat treatment and longer service life than medium-carbon steels. High-carbon steels have superior surface hardness resulting in high wear resistance. The AISI designations for High-carbon steel are: AISI 1055-1095, 1137-1151, and 1561-1572.

Monel 400
Monel 400: A combination of approximately 68% nickel and 28% copper. This alloy has superior resistance to oxidation and corrosion and provides excellent service in sea or brackish water.

Hastalloy C
Hastalloy C: A combination of nickel, chromium, molybdenum, iron, and tungsten. This alloy performs well at elevated temperatures in the range of 1600º to 1800º Fahrenheit (871°C to 982°C).

Titanium
Titanium: A silvery metallic chemical element; very malleable and ductile. Its principal function has been as an addition to steel to create steel alloys, but now is being used in its pure form (100% titanium) because of its high strength, light weight, and good corrosion resistance.

2.1.2.5 Conclusion

Due to their low heat resistance and limited resistance to solvents, PVC-U and PE cannot be used for the construction of the CI bioreactor in which a heating element of over 60°C is present. Even though PP is the most suitable polymer for the CI bioreactor construction at slightly elevated temperature, care should be taken in selecting the potential chemical disinfectant. Therefore, stainless steel is the preferred choice.

For the photobioreactors, polymers are also not suitable. Transparant PVC exists but becomes mat in time. It looses its transparency. Furthermore, the photobioreactors will be illuminated with halogen lamps, that convert 90% of the energy into heat. Polymers should be heat resistant in such a case. This is especially not possible for transparent PVC. Finally, as micropollutants will be investigated within BELISSIMA, polymers become less interesting as some of them contain significant amounts of additives. Therefore, glass and stainless steel are the only relevant options.

2.1.3 Overview of sterilisable commercial bioreactors

The way in which cleaning and sterilisation will be performed strongly depends on:
- ✓ Reactor volume;
- ✓ Reactor illumination;
- ✓ Material choice.

Commercially available bioreactor systems are either:

- ✓ Autoclavable or
- ✓ in-situ-sterilisable.

Commercially available options:

1. *Autoclavable bioreactors:* these are "benchtop" bioreactor for which the reactor vessel is cilindrical and made of glass. All glass bioreactors are autoclavable but not in situ steam sterilisable for safety reasons. Therefore, the commercially available working volumes (of double jacketed glass bioreactors) are limited to the range of 1 to maximally 10 l, which still allows manipulation for autoclaving. The reactor vessel is either single or double jacketed depending on the temperature control. For all systems, the reactor vessel is closed at the top with a stainless steel flange system on which several ports are foreseen for mounting the required sensors, for sampling, for supply or removal of medium, gas supply, etc. The bioreactor contains a stirrer with a motor placed on the top or at the bottom, depending on the reactor type. All bioreactor parts can be easily dismounted for cleaning and sterilisation.
   Bioreactors illuminated with halogen lamps are not commercially available. Illumination will have to be provided separately and will have to be integrated in the bioreactor system. Commercial photobioreactors either have a central light source and use a reflecting material on the inner vessel wall. For small reactor volumes up to 3 l, they use external illumination. Commercial systems use LED's, fluorescent lamps or hollow fibres as light source.
   Feed, acid and base dosing is achieved through peristaltic pumps. However, other types of systems are available.
   For all bioreactors the devices and instrumentation are connected to a central control which steers the bioreactor. Additional external devices and instrumentation can be coupled to the central control and can as such be integrated in the global process.

2. *In-situ-sterilisable bioreactors:* a distinction can be made based on the combination of materials used (combination glass/stainless steel (SS) and SS/SS). Laboratory-scale bioreactors are available from a working volume of 2 l up to ca. 20 – 30 l.

   Combination glass/SS:
   In this combination, the working volumes are limited from 2 l to ca. 15 l for safety reasons. The bioreactor consists of a lower part made of SS, which contains different ports for sensors, sampling ports, connections for steam sterilisation, etc… At the top a SS flange system is present which contains additional ports. A glass cilindrical column is clamped in between the SS bottom and top part. The height of the column depends on the desired working volume. The reactors can be cleaned in-situ.
   These reactors are used when visual inspection or follow-up is desired, but could also be applied as photobioreactor with external illumination, provided that the illumunated zone is sufficiently large compared to the dark zone of the SS bottom and top parts.

Combination SS/SS:
This combination differs from the previous one in that the glass column is replaced by a SS one. This allows for larger working volumes up to 20 – 30 l. These systems cannot be used as photobioreactors. However, a commercial system, in which the reactor medium is circulated outside the reactor vessel, is illuminated externally and then recycled to the dark bioreactor. This is a so-called circulation reactor in which the medium is continuously cycled between an illuminated and dark zone. The light sources are fluorescent lamps.

These reactor systems have the same possibilities for dosing, coupling of external devices and instrumentation as mentioned for the previous ones.

Suppliers:

- ✓ Autoclavable bioreactors: Bioengineering, Sartorius, New Brunswick, Applikon;
- ✓ Autoclavable photobioreactors: Bioengineering, Sartorius, New Brunswick;
- ✓ In-situ sterilisable bioreactors:
    1. Combination glass/SS: Bioengineering, Sartorius;
    2. Combination SS/SS: Bioengineering, Sartorius, New Brunswick, Applikon.
    3. Photobioreactors: Bioengineering, Sartorius;

All commercial available bioreactors are autonomous working devices, with their dedicated steering and control functions. They are independent of external control loops for their proper process. Signals from external devices or measurements could be integrated in the specific process of a bioreactor.
Signals from internal measurements or alarms can on the other hand be exported in an analog or digital way for further visualisation or certain actions.
Digital inputs finally could integrate certain external actions and generate actions like a switch off or stand-by mode of the bioreactor.

How a selection of photoreactors was made, is documented in 2.2.

2.2 Sizing of loop

The sizing of the loop was an iterative process. In summary it proceeded through the following steps:

- calculation of mass balances using the overall loop simulator and estimation of relative reactor volumes
- first round of contacts with suppliers of commercial hardware
- iteration of mass balance calculations
- first sizing of the complete loop for the BELISSIMA scenario
- reevaluation of the requirements (criteria document ESA)
- additional critical issues
- iteration of sizing for complete loop
- second round of contacts with suppliers of commercial hardware (see also TN80.24)
- final sizing of the complete loop (described in TN80.24)

2.2.1 Calculation of mass balances using the overall loop simulator and estimation of relative reactor volumes

A simulation on the BELISSIMA loop was performed by UBP (Annex 1), based on the following mission scenario:

- ✓ production of 40% of food of 6 crew members daily diet;
- ✓ treatment of non-edible parts of plants;
- ✓ production of 6 equivalent men of oxygen per day;
- ✓ production of 6 equivalent men of drinking water per day;
- ✓ recycle of 6 equivalent men of faecal material, urine and CO_2;
- ✓ if N from urine is too high the overall load should be undersized or the percentage of urine in the overall load lowered.

The equivalent composition of the waste and the load of CI are given in Table 5. The information concerning the faecal material is taken from TN45.3. The feeding of non-edible parts of plants is calculated based on data from TN32.3 and TN51.2. The amount of non-edible parts of plants is equaly divided over the three vegetables that will be used for feeding of the BELISSIMA loop. The amounts of waste produced are based on calculations for men.

Table 5: Composition of the waste in the BELISSIMA study

Material	Production / consumption of one man per day	Production / consumption of the 6 men crew	DM	Fresh load
Wastes		*Production of 6 equivalent man*		
- Fecal material	30 g DW/d	180 g DW/d	33%	545 g/d
- Urine	51 g DW/ d in 1,5 L/d	306 g DW/d in 9 liters	3,4%	9 l/d
- Toilet paper	18 g DW/d	108 g DW/d	100%	108 g/d
Vegetables		*40 % of food of 6 crew members daily diet*		
- Lettuce	270 g DW/d	648 g DW/d	5%	13 kg/d (~52 crops)
- Red beet	270 g DW/d	648 g DW/d	8%	8,1 kg/d (~34 crops)
- Wheat straw	270 g DW/d	648 g DW/d	100%	648 g/d

The total Dry Matter (DM) for this scenario is 2538 g/d. A dry matter concentration of 21 g/l in the influent (as for MELiSSA) should be maintained. This means that the flow of influent is around 120 liters per day.

A simplified simulation done by UBP resulted in hydraulic retention times (HRT) and compartment volumes as given in Table 6.

Table 6: Hydraulic retention times and relative reactor volumes for BELISSIMA (UBP simulation)

	HRT (days)	Volume (liters)
CI	10	1086
CII	3,0225	299
CIII	0,48227	47,9
CIVa	0,8288	82,0

The size of CIVa is rather small compared to the photobioreactor CII. In the MELISSA Pilot Plant the sizes of both photobioreactors are comparable. Having two photoreactors of similar size would also be advantageous for BELISSIMA in view of hardware harmonization and cost reduction. Therefore, it was agreed to aim for similar sizes for compartments II and IVa. For further calculations a dilution rate of 0,01 h^{-1} (HRT of 4,1667 d) was therefore used for CIVa.

The sizing exercise should further take into account the required sample volumes for general follow-up of the reactors and for study of the specific BELISSIMA microcompounds:

- ✓ standard follow-up of reactors: ~100 ml effluent per compartment per day
- ✓ BELISSIMA study: maximal 2 liters effluent per compartment per week

As a consequence, a sampling of **0,5 l/d** per compartment should be sufficient during intensive sampling campaigns. This can be reduced down to **0,1 l/d** during the intermediate periods. This means that in closed loop operation larger active volumes can be maintained within the reactors when sampling volumes are low. Alternatively, the compartments will always be operated at the same active volume and at a constant withdrawal of liquid volume. As suggested in TN80.15 the sampling volume should not exceed 15% of the flow rate of the specific compartment, in case of closed loop operation.
All these numbers result in the reactor volumes given in Table 7 and Table 8.

Table 7: Reactor volumes in case of intensive sampling campaigns

Compartment	Effluent samples	Calculated feeding	HRT (UBP)	Reactor volume
	l/d	l/d	days	liter
CIVa	0,5	3,3	4,1667	**13,8**
CIII	0,5	3,8	0,48227	**1,8**
CII	0,5	4,3	3,0225	**13,0**
CI	0,5	4,8	10	**48,0**

Table 8: Reactor volumes during intermediate periods

Compartment	Effluent samples	Calculated feeding	HRT (UBP)	Reactor volume
	l/d	l/d	days	liter
CIVa	0,1	4,5	4,1667	**18,8**
CIII	0,1	4,6	0,48227	**2,2**
CII	0,1	4,7	3,0225	**14,2**
CI	0,1	4,8	10	**48,0**

The simulation further indicated a shortage of CO_2 for compartment IVa. With the available amount, only 17% of nitrate can be assimilated. To achieve 100% removal, CO_2 supply is necessary and the associated reactor volume will then also be about 5 times higher.

2.2.2 First round of contacts with suppliers of commercial hardware

With the volumes presented in Table 7 and Table 8 in mind, various suppliers of commercial bioreactors were contacted. These were Bio-Engineering, Sartorius, New Brunswick and Applikon. The discussions mainly focussed on compartment II at this stage. They gave a first impression of the available options as well as of the open questions from the side of the suppliers.

2.2.3 Iteration of mass balance calculations

It appeared that the simulation program could only be used to obtain an order of magnitude for the liquid volumes of the different compartments and to identify major inconsistencies or shortages, such as the CO_2 shortage for complete nitrate elimination in compartment IVa. It cannot be used for detailed sizing and cannot take into account liquid losses due to sampling requirements.

For the first mass balance calculations several assumptions were made. The most critical one turned out to be the degradation efficiency of around 30% for the organic matter entering CI. Because of the new mission scenario developed for BELISSIMA, the composition of the feed to CI is different from MELiSSA and there is uncertainty on the degradation efficiency. If the efficiency was as high as 60%, the amount of volatile fatty acids produced would double and this would have serious implications on the downstream part of the loop. Such condition was recalculated and the outcome was used as a worst-case scenario for the iterative sizing of the complete loop as described in paragraph 2.2.6.

2.2.4 First sizing of the complete loop for the BELISSIMA scenario

The first attempt to perform a detailed sizing consisted of Photosim simulations for compartment II and IVa. The approach was the following. Based on known productivities, the optimal volume of the photoreactor is first calculated for a specific commercial system with given height and diameter and at a given light flux. Then, taking into account predetermined residence times, the associated liquid flow and substrate and biomass concentrations are calculated. For CII the residence time should be around 1 d, for CIVa around 3 d to obtain correct and controllable functioning. The smaller the dark zone in the photoreactor, the wider the operational range and the more operational flexibility.

The requirement to operate in axenic conditions is particularly stringent for the BELISSIMA study because one of the aims is to evaluate genetic stability and axenicity. In this respect, preference should be given to steam sterilizable equipment. However, the information

provided by the suppliers, indicated that all steam sterilizable reactors in the envisaged size range consist of a combination of glass and stainless or stainless steel (see 2.1.3) and had a dark volume of around 40%. This corresponds to a large dead zone for the productivity of the CII and CIVa photoreactors but more importantly, could be responsible for instabilities and physiological deviations in specific conditions in CII. In other words, photoreactors with a very small dark zone are preferred to ensure sufficient control in the operating engineering domain. Taking into account that the main risk of microbial contamination is related to feeding of the reactors and to transfer of liquids between compartments rather than to the bioreactors themselves, it was then decided to focus on autoclavable reactors with 100% illuminated volume and evaluate their operational flexibility.

Characteristics of commercial 5 and 10 l autoclavable bioreactors were collected and simulations performed. Because the commercial 10 l reactors had larger diameters than the 5 l ones which reduced their operational flexibility, a hypothetical photoreactor with a 10 l volume and a smaller diameter of 16 cm was included in the analysis as well. Keeping in mind the uncertainty on the degradation efficiency of the feed to CI, fibers degradation efficiency was chosen to be 20 and 50%. The simulation results for all these conditions are provided in Table 9 and Table 10.

The 5 l commercial systems can remove 13-14 g VFA/d at a maximum light intensity of 300 W/m². At 20% fiber degradation efficiency this corresponds to a mass flow of around 45 g feed/d to compartment I. At a nominal light flux of 75 W/m², this reduces to between 26 and 30 g feed/d. With nominal residence times between 9 and 34 h, the VFA input concentration to CII will vary between 0.55 and 2.2 g/l. Commercial 10 l systems perform relatively worse because they have larger diameters. The most flexible system is the one from Sartorius, with the smallest diameter. Still, at a maximal light intensity of 300 W/m², it can only remove 22 g VFA/d as compared to 14 g VFA/d for the best 5 l reactor (Applikon). The input mass flow rate to compartment I is 46 g/d at 20% fiber degradation efficiency and at 75 W/m². The feed concentration to compartment I can be varied between 1.7 and 6.6 g/l to achieve a VFA concentration range between 0.5 and 2 g/l.

At 50% fiber degradation efficiency, the input mass flow rate to compartment I can evidently be reduced to obtain the same VFA concentrations. In this case, the commercial 5 l systems can work with a feed concentration to CI between 1.2 and 4.5 g/l and a resulting VFA input concentration to CII of between 0.55 and 2.2 g/l. For the Sartorius 10 l system, the feed concentration to CI can range between 1.1 and 4.3 g/d.

The highest productivities could be achieved with a custom-made photoreactor, combining a 10 l volume with a diameter of 16 cm. In this case the input mass flow rate into the loop would amount to 85 g/d and 55 g/d at 20% and 50% fiber degradation efficiency respectively. The resulting feed concentrations into CI would be 2.1-6.5 g/l and 1.35-4.2 g/d respectively. VFA input concentrations to CII would be 2.1 g/l at maximum.

Table 9: Calculations for commercial photoreactors and a hypothetical one assuming 20% of fibers degradation efficiency in CI.

	APPLIKON		SARTORIUS BBI		NEW BRUNSWICK		HYPOTH
	V = 5.4 L D = 16 cm	**V = 10 L D = 24 cm**	**V = 5 L D = 16 cm**	**V = 10 L D = 19 cm**	**V = 5.5 L D = 17.6 cm**	**V = 10.5 L D = 21.1 cm**	**V = 10 L D = 16 cm**
Maximum performance of the PBR at q_0 = 300 W/m^2 (g_{VFA}/d)	14	18	13	22	13	21	26
Corresponding input mass flow rate (feed) in the loop (g_{TOT}/d)	**46 (1/9 man)**	**59 (1/7 man)**	**43 (1/10 man)**	**72 (1/6 man)**	**43 (1/10 man)**	**69 (1/6 man)**	**85 (1/5 man)**
! All following data are for nominal light flux at 75 W/m^2 !							
Nominal performance of the PBR at q_0 = 75 W/m^2 (g_{VFA}/d)	9	11	8	14	8	13	16
Corresponding input mass flow rate (feed) in the loop (g_{TOT}/d)	**30 (1/14 man)**	**36 (1/12 man)**	**26 (1/16 man)**	**46 (1/9 man)**	**26 (1/16 man)**	**43 (1/10 man)**	**52 (1/8 man)**
Nominal residence time in the PBR (h)	20	21	20	21	21	21	20
Nominal biomass concentration in the ouput of CII (g/L)	1,3	0,9	1,3	1,1	1,2	1	1,3
Nominal VFA concentration in the input of CII (g/L)	1,4	1	1,4	1,25	1,35	1,1	1,4
Nominal liquid flow rate (L/d)	6,5	11,5	6	11,4	6	12	12
Nominal resulting concentration of the feed (g/L)	**4,6**	**3,1**	**4,3**	**4**	**4,3**	**3,6**	**4,3**
Minimum residence time in the PBR (h)	9,5	9,5	9,5	9	9	9	9,5
Minimum biomass concentration in the ouput of CII (g/L)	0,55	0,44	0,55	0,45	0,5	0,4	0,55
Minimum VFA concentration in the input of CII (g/L)	0,65	0,44	0,65	0,5	0,55	0,45	0,65
Maximum liquid flow rate (L/d)	13,6	25	12,6	27	14,6	28	25
Minimum resulting concentration of the feed (g/L)	**2,2**	**1,44**	**2,1**	**1,7**	**1,8**	**1,5**	**2,1**
Maximum residence time in the PBR (h)	30	33	30	34	34	34	30
Maximum biomass concentration in the ouput of CII (g/L)	2	1,4	2	1,8	2	1,6	2
Maximum VFA concentration in the input of CII (g/L)	2,1	1,5	2,1	2	2,2	1,8	2,1
Minimum liquid flow rate (L/d)	4,3	7,3	4	7	3,8	7,4	8
Maximum resulting concentration of the feed (g/L)	**7**	**4,9**	**6,5**	**6,6**	**6,8**	**5,8**	**6,5**

Table 10: Calculations for commercial photoreactors and a hypothetical one assuming 50% of fibers degradation efficiency in CI.

	APPLIKON		SARTORIUS BBI		NEW BRUNSWICK		HYPOTH
	V = 5.4 L D = 16 cm	V = 10 L D = 24 cm	V = 5 L D = 16 cm	V = 10 L D = 19 cm	V = 5.5 L D = 17.6 cm	V = 10.5 L D = 21.1 cm	V = 10 L D = 16 cm
Maximum performance of the PBR at q_0 = 300 W/m^2 (g_{VFA}/d)	14	18	13	22	13	21	26
Corresponding input mass flow rate (feed) in the loop (g_{TOT}/d)	**30 (1/14 man)**	**38 (1/11 man)**	**28 (1/15 man)**	**47 (1/9 man)**	**28 (1/15 man)**	**44 (1/10 man)**	**55 (1/8 man)**
! All the following data are for nominal light flux at 75 W/m^2 !							
Nominal performance of the PBR at q_0 = 75 W/m^2 (g_{VFA}/d)	9	11	8	14	8	13	16
Corresponding input mass flow rate (feed) in the loop (g_{TOT}/d)	**19 (1/22 man)**	**23 (1/18 man)**	**17 (1/24 man)**	**30 (1/14 man)**	**17 (1/24 man)**	**28 (1/15 man)**	**34 (1/12 man)**
Nominal residence time in the PBR (h)	20	21	20	21	21	21	20
Nominal biomass concentration in the output of CII (g/L)	1,3	0,9	1,3	1,1	1,2	1	1,3
Nominal VFA concentration in the input of CII (g/L)	1,4	1	1,4	1,25	1,35	1,1	1,4
Nominal liquid flow rate (L/d)	6,5	11,5	6	11,4	6	12	12
Nominal resulting concentration of the feed (g/L)	**2,9**	**2**	**2,8**	**2,6**	**2,8**	**2,3**	**2,8**
Minimum residence time in the PBR (h)	9,5	9,5	9,5	9	9	9	9,5
Minimum biomass concentration in the ouput of CII (g/L)	0,55	0,44	0,55	0,45	0,5	0,4	0,55
Minimum VFA concentration in the input of CII (g/L)	0,65	0,44	0,65	0,5	0,55	0,45	0,65
Maximum liquid flow rate (L/d)	13,6	25	12,6	27	14,6	28	25
Minimum resulting concentration of the feed (g/L)	**1,4**	**0,9**	**1,35**	**1,1**	**1,2**	**1**	**1,35**
Maximum residence time in the PBR (h)	30	33	30	34	34	34	30
Maximum biomass concentration in the ouput of CII (g/L)	2	1,4	2	1,8	2	1,6	2
Maximum VFA concentration in the input of CII (g/L)	2,1	1,5	2,1	2	2,2	1,8	2,1
Minimum liquid flow rate (L/d)	4,3	7,3	4	7	3,8	7,4	8
Maximum resulting concentration of the feed (g/L)	**4,4**	**3,1**	**4,2**	**4,3**	**4,5**	**3,8**	**4,2**

Currently, compartment I of the MELiSSA loop is operated at a feed concentration of 20 g/l and generates around 5 g/l of VFA. Even though the MELiSSA feed is different from the BELISSIMA one in the sense that other relative contributions from vegetables and fecal material are used and that urine is an input in BELISSIMA and not in MELiSSA, it is not expected that the VFA production would be lower, although there is an uncertainty about the exact value. This means that there is a discrepancy between the VFA output of CI as it is operated now and the required input to CII. There are two ways to solve this. Either the feed to CI is diluted, or the output of CI is diluted into the proper VFA input range for CII. Dilution of the feed to CI has never been tested. So far, CI has always been operated at feed concentrations around 20 g/l. What the effect of diluted feed will be on the VFA output is hard to predict.
The other option would be to operate CI at the usual 20 g/l feed concentration and to dilute the effluent. Based on the above calculations, a five- to tenfold dilution would probably be needed. This would bring the VFA concentration in the appropriate range, but would also dilute the microcompounds or their metabolites and these are of prime importance for the BELISSIMA study.
Both scenarios have the drawback that they lower the concentration of microcompounds in the loop. Let us perform the calculations for the example of hormones. As estimated in TN80.12, the excreted amount of oestrogens amounts to 12-182 µg/person/d, of synthetic hormone 9-32 µg/person/d and for androgens 5000-16000 µg:l. Sincœ the female hormones occur in the lowest concentration in urine, this can be considered a worst-case scenario. Assuming an urine production of 1.5 l/person/d, the estimated concentrations of the natural and synthetic hormones are 8-121 and 6-21 µg/l respectively.

Scenario 1: dilution of effluent CI
- estimated feed concentration when feed to CI is prepared at 20 g/l (9 l urine in 120 l of feed slurry): 0.6-9 and 0.5-2 µg/l of natural and synthetic hormones respectively
- estimated concentration in effluent CI assuming 90% degradation efficiency: 0.06-0.9 and 0.05-0.2 µg/l respectively
- estimated concentration in feed CII after fivefold dilution of effluent CI: 0.01-0.18 and 0.01-0.04 µg/l respectively.

Scenario 2: dilution of feed CI
- estimated feed concentration when feed to CI is prepared at 4 g/l: 0.1-1.5 and 0.1-0.4 µg/l respectively
- estimated concentration in effluent CI assuming 90% degradation efficiency: 0.01-0.15 and 0.01-0.04 µg/l respectively
- estimated concentration in feed CII: 0.01-0.15 and 0.01-0.04 µg/l respectively.

The detection limit for determination of female hormones through YES- or MLVN-tests is 3 and 0.1 ng/l of E2 (17β-estradiol) when 1 l samples are available. Hence, analysis of the hormones is still possible at the required dilution rate.

Dilution of the feed or dilution of the effluent of compartment I does not make any difference in terms of hormone concentrations at the entrance to CII. However, we cannot be certain that

the transformation processes in CI are similar under both conditions. Taking into account that CI operation is now well described for the MELiSSA scenario at 2% solids content of the feed, we propose CI feeding with a 2% slurry in BELISSIMA as well. To eliminate the discrepancy between the VFA concentration produced by CI and the required input concentration for CII, a dilution of the effluent of CI will be performed.

2.2.5 Additional critical issues

2.2.5.1 Compartment IVb

The BELISSIMA loop will not contain a Higher Plant Compartment (HPC) in the first phases, but must be designed in such a way that a HPC can be included in the future. For statistical purposes the HPC should be large enough to produce sufficient vegetables for replicate analyses. In this case, the HPC should be about 2 m^3. The first calculations on N content of the liquid flow in the BELISSIMA loop result in a 60 mg N/l concentration for a flow of 6 l/d. If the full flow was directed to a HPC, then this would probably support 1 to 3 m^2, which is in the range mentioned above. The outlet of CIII should therefore not be split between CIVa or CIVb but should be sent to either of them.
This topic will be further discussed in TN80.24 after finalisation of the sizing exercise.

2.2.5.2 Sampling in Compartment III

Ideally, there should be a possibility to perform biomass sampling at various heights in compartment III. This would allow to investigate the relative microbial composition of the biofilm over the reactor height. However, due to the high risk of contamination and the danger of disturbing flow patterns through the packed bed system after removal of some carrier material, it was decided not to perform such samplings during test runs, but only at the end of a test run. This implies that no provisions for sampling at different heights have to be taken in the design of the compartment.

2.2.5.3 Material selection

The conclusions of 2.1.2.5 already indicated that the preferred material for the bioreactors would be glass or stainless steel. For the rest of the loop, material selection is equally important. Various reasons argue against the use of plastic. It may leach microcompounds which may interfere with the target microcompound analysis. After several sterilization cycles, gas leaks can occur in closed tanks. Hence, plastic should preferably not be used. The use of glass containers as influent and effluent tanks is not desirable. At volumes above 10-15 l, as required for the influent and effluent buffers, they are not steam sterilizable. Frequent autoclaving would also present a risk of breaking the tanks and is also limited in terms of tank size. The use of stainless steel will inevitably result in the release of metals. To prevent biofilm formation on the surface of reactors and tanks, polishing of INOX to reduce the surface roughness is preferable. Polishing down to Ra 0.8 is often used in the food industry, and to 0.4 in the pharmaceutical industry. Going to even lower Ra values has very high cost implications. Furthermore, it does not make sense when it is not even used in pharmaceutical industry.

In case of steam sterilization, polished INOX tanks will be selected. Otherwise, glass tanks are suited as well.

2.2.5.4 Sterility and axenicity

From the first contacts with suppliers, the use of sterile coupling systems was suggested. ESA however has bad experience with them and prefers in-line steam sterilizable connections and buffer tanks.

2.2.5.5 Illumination

Compartment II and IVa are photobioreactors. Because of the high heat dissipation, the use of halogen lamps is disadvantageous. However, since the suppliers cannot provide LEDs with the same spectrum as the halogen lamps used so far, it was decided to stick to halogen illumination.

2.2.5.6 Remaining macronutrients in the loop

To avoid nitrate limitations in compartment IVa, nitrate will not be completely exhausted and some remaining nitrate will be present in the liquid. In open loop, this is not a problem, but upon loop closure, nitrates might progressively accumulate. To avoid this, a selective nitrate removing technique could be incorporated, such as a resin or a biological treatment step; however, this would certainly also affect the microcompound composition of the liquid loop which is not desirable. Since it is currently not possible to calculate the exact value of the nitrate concentration in the output of CIVa, this problem will be addressed in detail once the complete loop is in operation and correct values have been measured. Similar problems might have to be covered for sulphate and phosphate.

2.2.6 Iteration of sizing for complete loop

With the above decisions, the design of the complete loop was refined. The boundary conditions were:

- The feed to CI has a dry matter content of 21 g/l and the hydraulic retention time is 10 d.
- The CI effluent is diluted before being sent to CII.
- The degradation efficiency in CI is set at 50%. This will be the worst case scenario in terms of VFA production. If the efficiency is lower, less VFA will be produced and dilution of the CI effluent will be lower.
- CII has a 10 l volume. This gives some flexibility in terms of sampling volumes. Two cases are considered: a commercially available reactor with 24 cm diameter as a worst case and a reactor with 16 cm diameter which is not commercially available as a best case.

The approach used was the following.:

- first fixing the operating conditions for CII for both scenarios
- then backcalculating the CI volume based on the required dilution of its permeate

- calculation of the CIII and CIVa volumes.

This results in the designs presented in Figure 2 and Figure 3.

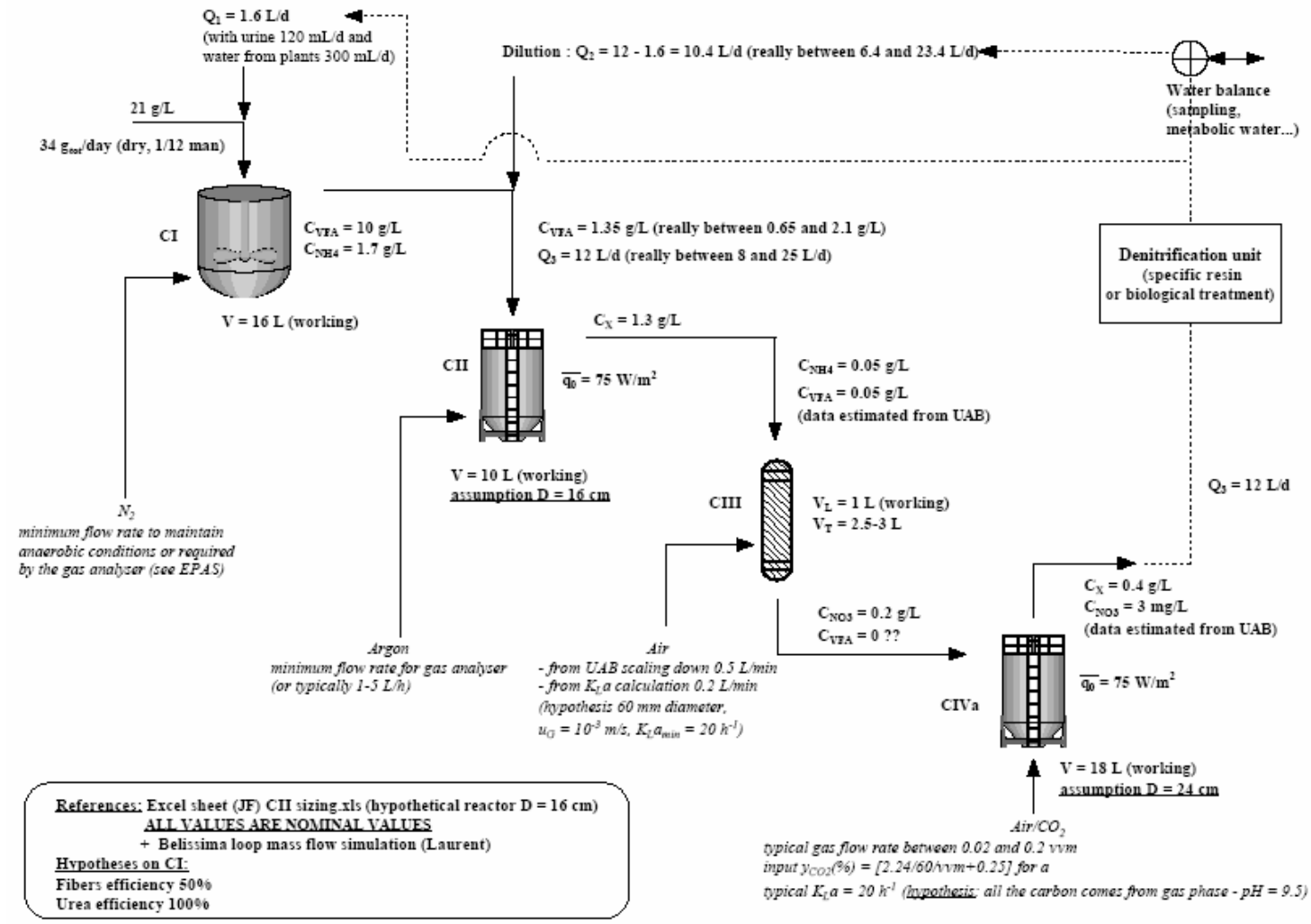

Figure 2: Design of BELISSIMA loop starting from a hypothetical photoreactor with a 16 cm diameter for CII (best case scenario).

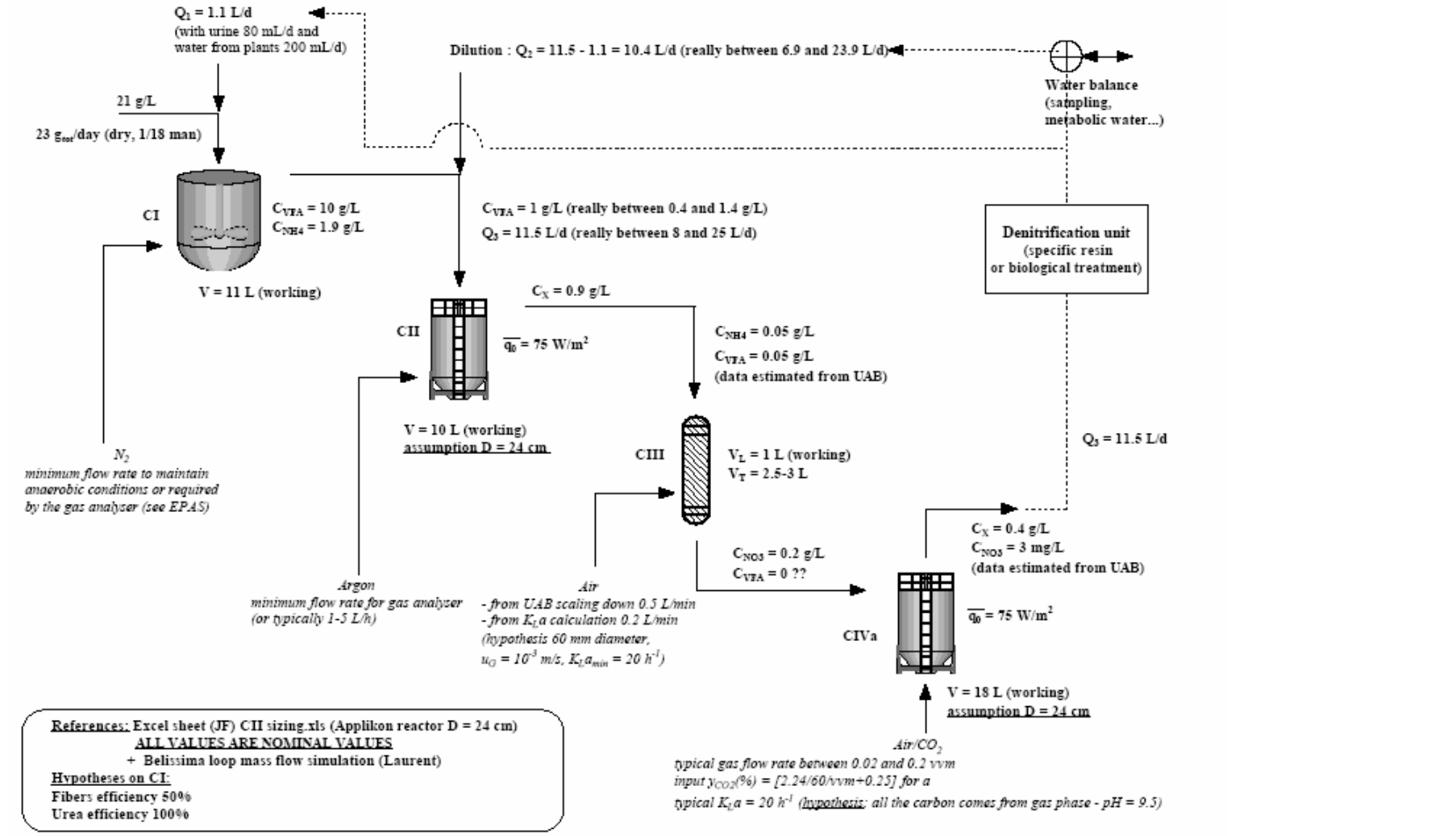

Figure 3: Design of BELISSIMA loop starting from a commercial photoreactor with a 24 cm diameter for CII (worst case scenario).

The presented schemes indicate a working volume range of between 11 and 16 l for CI, 10 l for CII, 1 l for CIII and 18 l for CIVa. Only the working volume of CI is affected by the selected hardware for CII. The calculations did not yet take into account that the liquid flow rate through the loop will gradually decrease due to sampling after each compartment.
If 0.5 l sample is removed after CI, this should be compensated by increasing the liquid flow to 2.1 l/d and, accordingly, increasing the volume to 21 l. Assuming a daily sampling of 0.5 l/d, the relative working volumes would therefore be approximately 21/10/1/16.

If the calculation was redone starting from a commercial 5 l photoreactor for CII instead of a 10 l one, the resulting working volumes would be 12/5/0.6/6-10 l respectively. This would be advantageous because an autoclavable commercial glass reactor could be used for CIVa as well. As mentioned before, autoclavable glass reactors do not exist at volumes of 18 l. Furthermore, the corresponding liquid flow of around 6.5 l/d would lead to smaller buffer tanks. This would facilitate maintenance and operation of the loop to a large extent.

2.2.7 Second round of contacts with suppliers of commercial hardware

With the relative volumes calculated above and starting from a 10 l working volume for the second compartment, various suppliers were contacted again: Bio-Engineering, Sartorius and Applikon. New Brunswick indicated that they could only provide standard hardware and could not supply the tailor-made equipment needed for BELISSIMA. EPAS works with a different supplier for the construction of compartment I.

The quotations from Bio-Engineering, Sartorius and Applikon focussed on CII and CIII and were used as a basis to select a supplier for the BELISSIMA hardware (excluding CI).

2.2.8 Final sizing of the complete loop

Taking into account sampling volumes of 0.5 l/d, the actual working volumes for compartments I, II, III and IVa are approximately 21 l, 10 l, 1 l and 16 l respectively.

3 Waste preparation unit

3.1 General

The substrate of CI contains particulate material. The size of the particles must be minimised to ensure an efficient hydrolysis of the substrate. The membranes that will be used are tubular membranes and have an internal diameter of 8 mm. This means that the size of the particles must be lower than 8 mm to prevent blockage of the membranes. A particle size smaller than 2 mm is the objective to avoid clogging of the inlet of the membranes. Particles are present under different shapes that result from the initial composition of the different wastes and the grinding method used. Grinded wheat for instance is made of fibrous particles of different sizes. Therefore the size criteria must be applied to the maximum length of one particle.
The substrate must be collected, treated, stored and fed in a homogeneous mixture in order to guarantee the influent quality used during the tests. Because of their difference of properties, the different materials used in the influent cannot be grinded by the same technology. Especially wheat straw, which is dry, needs a specific mill grinder and needs to be grinded separately from the other fresh materials. These fresh materials can possibly be grinded with water. Usually, finer particles can be obtained using smaller lab-scale techniques. These techniques have the disadvantage to be time-consuming. On the other hand, bigger-scale techniques allow some automation and treat bigger amounts of material, they consume therefore less time, but the particles size is often bigger. Therefore, a compromise needs to be made, depending on the amount of substrate that will need to be prepared, which is directly related to the bioreactor size.
It is proposed to build an intermediate waste preparation unit, semi-automated, for the grinding and mixing of fresh material (faecal material, lettuce, red beet) and toilet paper. The wheat straw will be grinded separately in a dedicated lab grinder and will then be added into the waste preparation unit. These solutions have been used successfully with the Compartment I from MELiSSA.

3.2 Principle of waste preparation unit

The substrate (faecal material, toilet paper, beet and lettuce) is introduced in a closed and semi-automated loop where an industrial kitchen disposer as well as a grinding pump are integrated. The grinding pump allows to maintain the recirculation flow through the mixing loop and also a rough grinding of fresh material. The fine grinding and homogenization of the total substrate is performed by the industrial kitchen disposer. By continuous recirculation of the substrate in this mixing loop, the particles size is aimed to be reduced smaller than 2 mm. The wheat cannot be processed efficiently directly through this loop, since its dry state and its fibrous and hard structure require different grinding techniques to be reduced. Therefore it is individually grinded by means of a cutting mill and afterwards introduced in the mixing loop. The material is diluted until the desired concentration (reached by automated addition of the appropriate amount of water) and then drained in a collection vessel to be fed to the influent tank. The faecal material is added frozen in the unit, in order to reduce the biological risks and the inconvenience related to faecal material manipulation.

Catering kitchens are equipped with similar apparatuses to process food waste present in rinsing waters. Material is entered together with water at the top of the machine and is cut by turning knives. The material is removed by the centrifugal force of the rotor on which the knives are mounted. By recirculation of the waste stream a particle size smaller than 2 mm can be obtained.

To ensure the circulation of wastes in the feed preparation loop, a progressive cavity pump equipped with a grinder is used. The pump is equipped with a macerator directly combined in its housing, which can reduce frozen faecal material. This type of macerators is mainly used for municipal and industrial wastes. They macerate the solid and fibrous components in the wastewater or sludge, increasing the operating safety and the service life of pumps and other machinery.

A system based on a similar concept has been used in the frame of MELiSSA. Several critical points have been pointed out:

- foam formation in the buffer tank: can lead to difficulties to measure liquid volume and to ensure homogeneity of influent
- cleaning step: time-consuming
- unease of waste introduction in the system.

The present concept and design were adapted in order to take into account these critical points and optimize the waste preparation step.

Prevention of foam formation and reduction of its consequences:

The inlet of influent coming from the industrial kitchen grinder is placed on the buffer tank R-W-01 under the liquid level (see Figure 4), in order to reduce turbulences and foam formation. However, the use of such a grinder (G-W-01) implies air suction and therefore it is not possible to prevent entirely foam formation, except by using chemical anti-foam agents, which is excluded in the frame of BELISSIMA.

To guarantee a proper volume detection, it is proposed to work with 2 resistance sensors instead of a classical level switch, which may give a wrong signal because of the foam.

The top of the buffer tank (R-W-01) will include a sealing to prevent foam overflowing.

Optimisation of cleaning:

A cleaning tank coupled with a pressure pump will allow to flush water and cleaning agent at an appropriate flow and pressure. Nozzles will be integrated in R-W-02, and in R-W-01 at the top and also at the level of the pump PMP-W-01. The use of automated valves will allow a semi-automated cleaning. These propositions should allow to optimize the cleaning and reduce the time needed for the operator.

Ease of the introduction of waste in the unit:

The operator must introduce the waste material in the unit: frozen lettuce, frozen red beet, frozen faecal material, grinded wheat straw, and toilet paper. This step needs to be as easy and short as possible for the user, and to reduce to the maximum the contact between user and waste material, and particularly faecal material.

The waste material will be introduced in the tank R-W-02 through a large funnel on the side. The top of the tank will be transparent and removable, in order to allow the user to check visually that the waste is well grinded through G-W-01 without risks of waste splashing. A

fixed stick will be used to push the waste through the inlet of G-W-01, when necessary. A rubber support will allow the user to push or pull the stick without taking it out of the tank, preventing contact with waste material and optimising the ease of unit operation.
This option is presented in Figure 4.
Another possibility is to use a screw feeder (Figure 5) to introduce the waste in the unit (automated, or possibly manual). In that case, the waste material will be first introduced, and the recirculation of material and water will be performed afterwards.

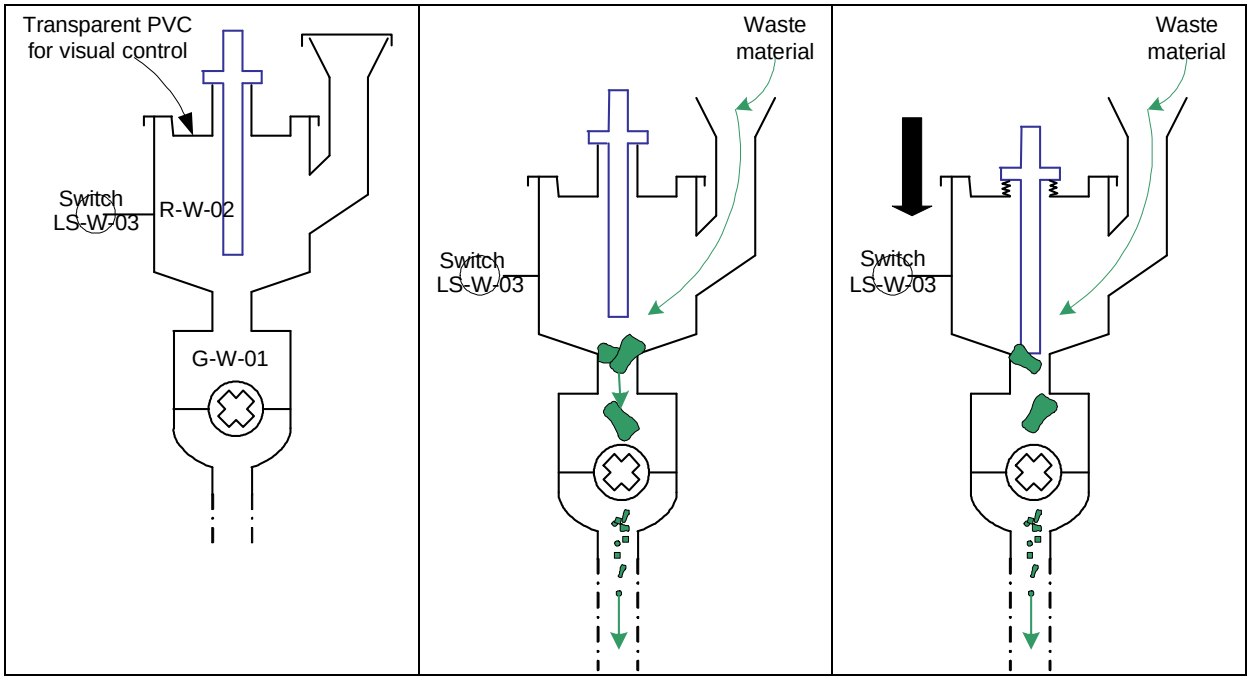

Figure 4: Principle of waste introduction in the unit (option 1)

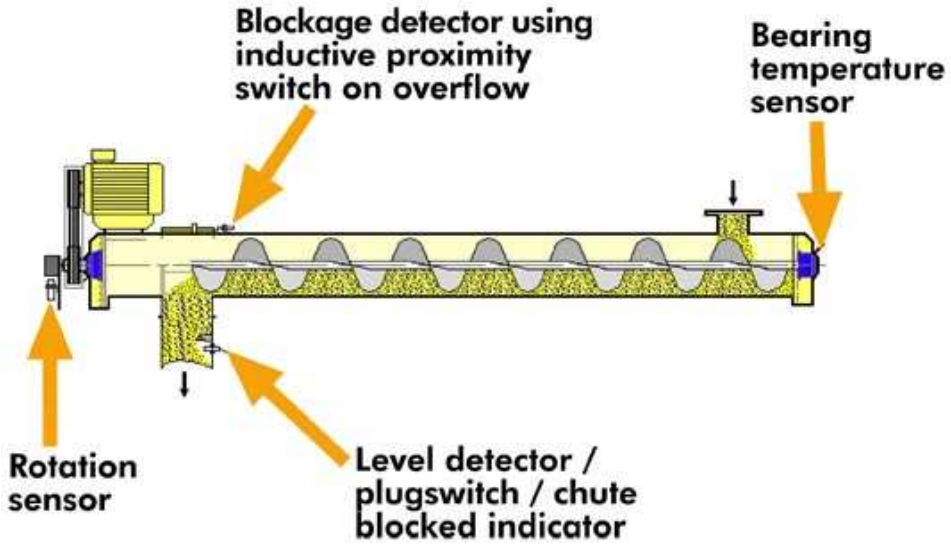

Figure 5: Principle of a screw feeder

3.3 Critical item: grinding frozen material

As mentioned above, a recirculation loop will be used to ensure sufficient reduction of particles size. The frozen material (lettuce, red beet, faecal material) and the toilet paper are first roughly grinded through an industrial kitchen grinder. Then a submersible grinding pump can be used for the continuous recirculation of the waste stream.

Industrial kitchen grinder

An In-Sink-Erator food waste disposer such as the Evolution Essential could be used. It includes two grind stages, which allow to grind difficult food waste. The 40-Ounce stainless steel grind chamber and grind component handle more volume than standard disposers. The specifications of this grinder are presented in Table 11.

Table 11: Specifications of the In-Sink-Erator Food Waste Disposer

Type of Feed	Continuous
On/Off Control	Wall Switch
Motor	Single Phase
HP	3/4
Volts	120
HZ	60
RPM	1725
Amp. (Avg. Load)	8.1
Time Rating	Intermittent
Lubrication	Permanently Lubricated Upper & Lower Bearings
Shipping Weight (Approx.)	20.8 lbs.
Unit Finish	Black Enamel Gray TEN-1706
Overall Height	12-5/8"
SoundSeal Technology	Anti-Vibration Mount™ Anti-Vibration Tailpipe Mount™ Quiet Collar™ Sink Baffle SoundLimiter™ Insulation
MultiGrind Technology	GrindShear Ring™
Grind Chamber Capacity	40 oz.
Motor Protection	Manual Reset Overload
Average Water Usage	1/4 Gallon Per Person Per Day
Average Electrical Usage	1/2 KWH Per Month
Drain Connection	1-1/2" Anti-Vibration (Hose Clamp)
Dishwasher Drain Connection	Yes

The grinding mechanism is shown in Figure 6.

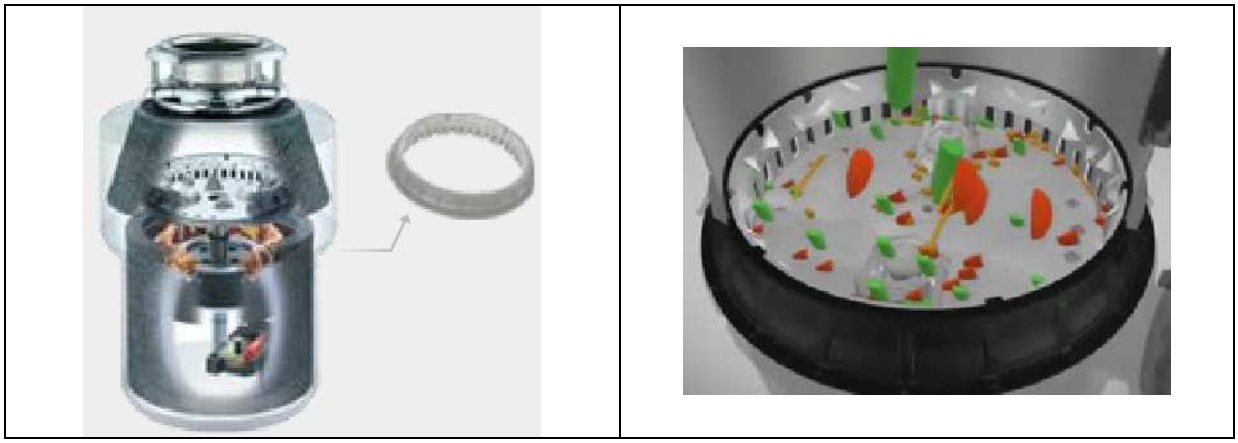

Figure 6: Grinding mechanism of In-Sink-Erator food waste disposer.

Submersible grinding pump

The DAB submersible pump grinder 2025T has been tested successfully in the past in the frame of EWC and it is proposed to work with a similar grinding pump.

It consists of a submersible cast iron pump with a triturator suitable for lifting or drainage installations for civil and industrial sewage. The material present in the sewage is reduced into small particles, thanks to the grinding system.

Figure 7: Submersible grinding pump

3.4 Critical item: grinding wheat straw

The wheat straw can be grinded in two different steps:

- First the straw pieces are roughly grinded into smaller pieces of 0.5 to 2.5 cm by means of a robot centrifugal grinder.

  The Cutting mill SM 100 from Retsch (Figure 8) could be used for this first size reduction step. It has already been tested with wheat straw. The processing of straw is very fast and it produces straw pieces from the right size to be fed to the a centrifugal grinder.

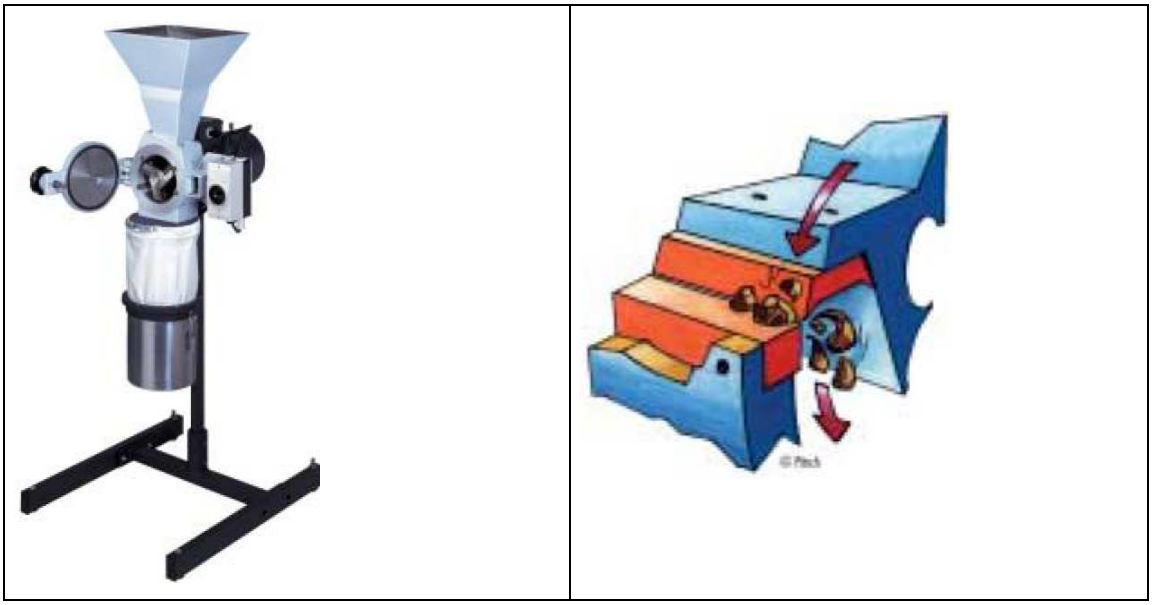

Figure 8: Picture and principle of the cutting mill for first grinding of wheat straw

- These pieces are then transferred to the centrifugal grinder Ultra Centrifugal Mill ZM100 from Retsch (see Figure 9) to produce fine powdered wheat. Centrifugal mills are used for fine grinding of soft to medium-hard, elastic, brittle and fibrous dry materials. Grinding takes place in the mill by the impact and shearing action between the rotor and the fixed ring sieve. The feed material passes through the funnel with splashback protection onto the rotor. With the centrifugal acceleration it is hurled outwards with great energy and is precrushed on the wedge-shaped rotor teeth before being finely ground between the rotor and the screen. An inversed sieve with holes of 2 mm was used to obtain fine powder with particles smaller than 1 mm.

  The feed material can be introduced manually or via a feed unit controlled as a function of load. The ground material is collected in a tray or in a paper filter bag via a passage receptacle.

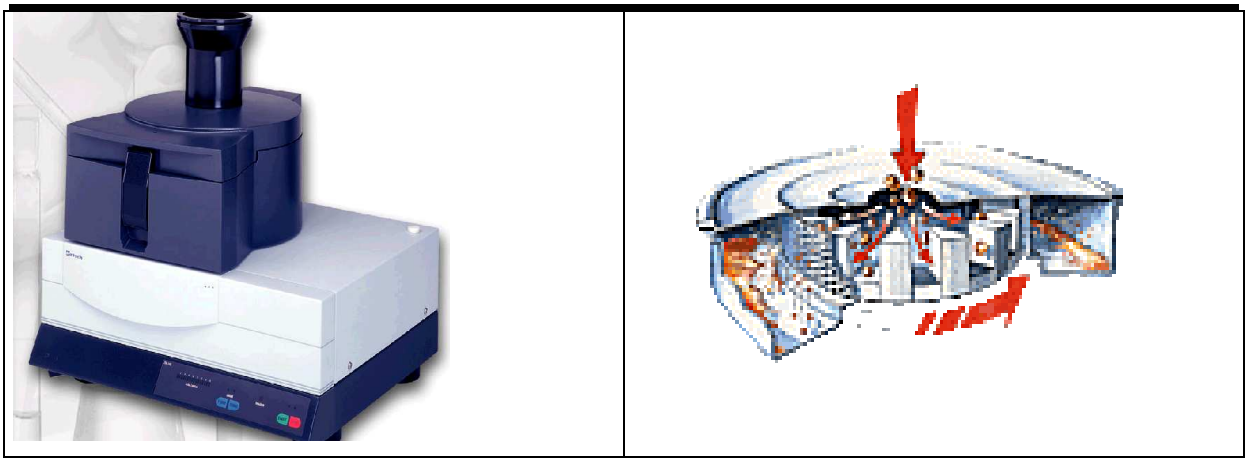

Figure 9: Picture and principle of the centrifugal lab grinder for wheat straw

Table 12 compares various grinders for the influent preparation system.

Table 12: Overview of grinders for the Influent Preparation System

	Straw:		Frozen material:	
Criteria / Requirement	Lab grinder straw 1st step	Lab grinder straw 2nd step	Inudstrial kitchen grinder	Submersible grinding pump
Ability to grind frozen material				
Lettuce			x	x
Red beet			x	x
Toilet paper			x	x
Faecal material			x	x
Ability to grind dry material				
Wheat straw	x	x		
Homogenous mixture	yes	yes	yes	yes
Material feed size	max 60 x 80 mm	Up to 10 mm	40 - 50 cm	
Final fineness (expected << 4mm)	0,25 - 20 mm	< 40 µm	< 3,15 mm	< 2 mm
Minimize any influent contamination	prevent heavy-metal contamination			
Minimize contact between user and influent (safety issues)	possibility to add dust filters			
Minimize operation time	yes	no! About 1 h/ 100g	yes	yes
Maximize automation level	manual operation	manual operation	automated	automated
Minimize price	9.319 €	5000€ ??	1.409 €	1.268 €
Brand/Model	Retsch Cutting Mill SM100	Retsch Ultra Centrifugal Mill ZM 200	B2085 Motor Kitchen grinder ISE	Pump RT 2015-2M
Tests	One test done by Retsch => satisfying	Tested at EPAS for EWC => result OK but time consuming	Tested at EPAS for EWC => satisfying	Tested at EPAS for EWC => satisfying
Delivery time	3-4 weeks	3-4 weeks		
Guarantee	2 years	2 years		

3.5 Concept scheme

A concept scheme is given in Figure 10.

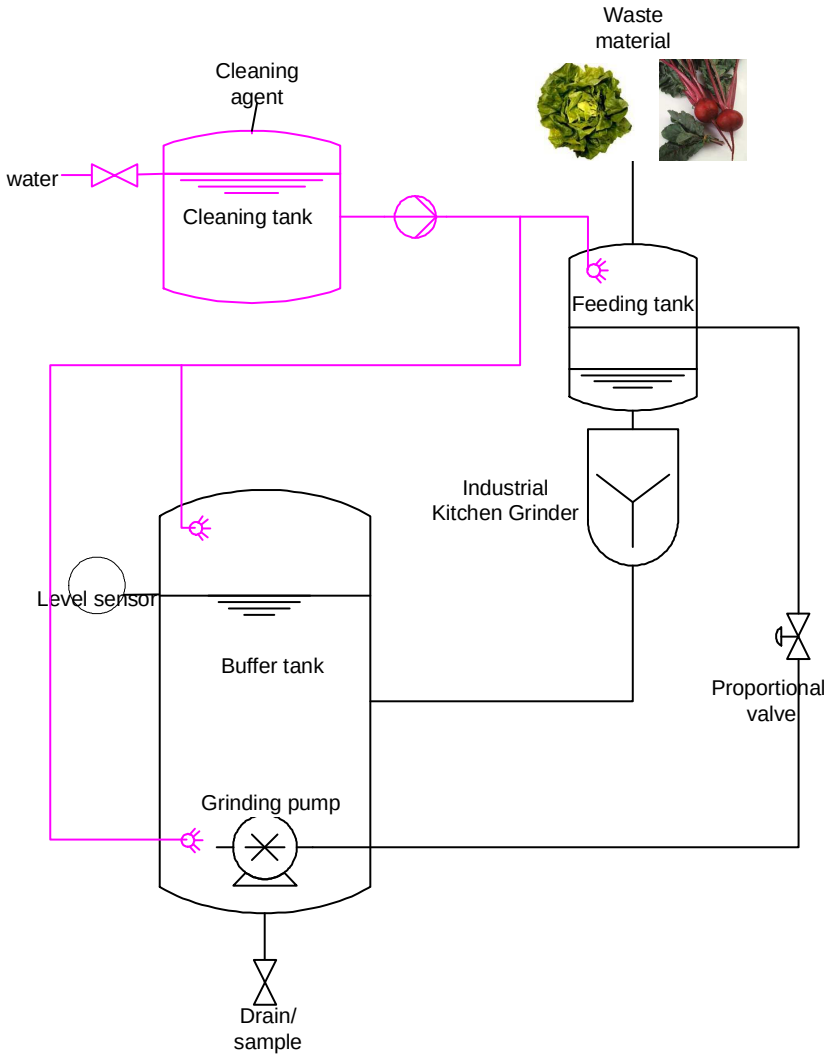

Figure 10: Concept of waste preparation unit

4 Compartment I

4.1 Summary of design requirements

The design requirements for this waste reactor were described in detail in TN80.16. Table 13 summarizes the technical requirements, related to the reactor itself.

Table 13: Summary of reactor and filtration unit (FU) requirements

	Detail	Requirements		Comment
		Reactor	FU	
Operation	Feeding	(semi-) continuous	continuous	
	Sterility	no	yes	
	Gas closure	yes		
	Pressure	100±20 mbar		slight overpressure, to avoid contamination
	pH-correction	yes		
	Gas flow	N_2		for anaerobic conditions and overpressure
Reactor	Size	~50 liters		
	Stirring	good homogeneity		
	Heating	yes		

Some general considerations for the design are given below:

Influent tank:
- ✓ inlet of N_2: with a backpressure regulator to compensate the pressure drop due to feeding by N_2;
- ✓ mechanical pressure safety (pressure relief valve);
- ✓ cooler (water bath) with double jacket;
- ✓ feeding is done in semi-continuous way, by means of a recirculation loop on the influent tank, and an automated valve equipped with a timer, that opens in the direction of the bioreactor at regular intervals of time;
- ✓ on-line measurements: pressure, temperature, volume;
- ✓ completely stirred (blender);
- ✓ liquid/gas sampling ports.

Bioreactor:
- ✓ automated pH correction with addition of acid/ base;
- ✓ mechanical pressure safety (pressure relief valve);
- ✓ warm water bath with double jacket;

- ✓ on-line measurements: pressure, temperature, volume, pH;
- ✓ completely stirred (blender);
- ✓ liquid/gas sampling ports.

Gas Loop:

- ✓ passive loop:
  - o Automated addition of N_2 when pressure drops (manual backpressure regulator)
  - o Automated evacuation of gas produced to columns when pressure increases (manual backpressure regulator)
- ✓ on-line analysis of the gas phase is highly recommended;
- ✓ gas production measured on columns.

Filtration Unit:

- ✓ ceramic ultra-filtration membrane, followed by dead-end filtration will be used to separate the soluble components and sterilize the filtrate;
- ✓ 2 parallel membrane modules to switch in case of clogging;
- ✓ cleaning and sterilization in place, automated, with stopping of filtration process

The materials are selected to not release contaminants in the compartment, to be easily cleaned and to be steam-sterilizable for parts of the Filtration Unit. Stainless steel 314 is selected for the bioreactor, tanks and main liquid tubes. Teflon is selected for gas tubes.

4.2 Process characteristics

4.2.1 Sizing

Several important aspects must be taken into account for the sizing of the compartment. Mainly, the mission scenario and the sampling volumes must be well investigated in order to estimate the necessary reactor volume.

In the MELiSSA CI, a total DM concentration of 21 g/L in the influent is used. The same concentration will be used in BELISSIMA, because there is no experience in the consortium on the use of a more diluted feed. With higher concentrations, the mixing and circulation by pumps of the influent is difficult because of the presence of plants particles. The scenario of BELISSIMA implies the feeding of about 2.5 kg/d of total dry matter. To use the same concentration, this means that the flow of influent fed should be about 120 L/d (including the 9 L of urine daily produced). The MELiSSA compartment I is operated with an hydraulic residence time (HRT) of 10 days. To keep this process parameter, this means that a bioreactor adapted for this mission scenario would need a volume of 1,2 m^3. It is proposed to work in proportional conditions with this scenario, at a smaller scale as defined by the sample needs.

According to TN80.16, about 500 mL of liquid sample will be needed per day. This should constitute no more than 10% of the liquid flow through the loop. At a HRT of 10 days this would mean a liquid volume of about 65 L for Compartment I. However, as detailed in 2.2.6 and 2.2.8, there is a need for dilution of the liquid flow after CI to make sure that the VFA

concentration is lowered to be compatible with CII. When the required liquid flow to CII is 12 l/d (see Figure 3), the outlet of CI should be around 1.6 l/d. taking into account additional sampling of about 0.5 l per day, the total liquid flow into CI must be 2.1 l/d. Hence, the required CI volume is around 21 l.

The CI will be equipped with an Influent tank and a Filtrate (effluent) tank. In nominal operation, the flow of influent and filtrate will be 2.1 L/d. It is proposed to work with an influent tank of 15 L liquid volume, to allow the filling of the influent tank only once a week. The filtrate tank volume should be as small as possible, because of the contamination and possible bacterial growth issues. A liquid volume of 5 L is proposed.

4.2.2 Functioning mode

It will be possible to operate the reactor in batch or continuous mode.

CI will operate under anaerobic conditions. Any air inlet will be completely avoided. N_2 inlet will be provided. All the equipment of the gas loop will be gastight.

In addition, the following modes will be taken into account in the control codes:
- ✓ Start up
- ✓ Shut down
- ✓ Changes in operation set points and control
- ✓ Alarms monitoring

4.2.3 Interfaces

Table 14 presents an overview of the interfaces related to the stand-alone operation of CI.

Table 14: General overview of the interfaces of CI

Interface (tag P&ID)	Description	Needed equipment
Influent tank		
I-V-01	Gas evacuation to the outside in case of overpressure in the influent tank	Emergency valve left unconnected to release gas as fast as possible
I-V-02	Inlet of fresh influent in influent tank	Multifix tube (EPTM)
I-V-03	Outlet of influent (drain) from influent tank	Multifix tube (EPTM) + mobile drain vessel
Bioreactor		
I-R-01	Outlet of reactor content (drain) from bioreactor	Multifix tube (EPTM) + mobile drain vessel
I-R-02	Gas evacuation to the outside in case of overpressure in the bioreactor	Emergency valve left unconnected to release gas as fast as possible
Filtration Unit		
I-F-01	Outlet of retentate (drain) from the	Multifix tube (EPTM) + mobile drain

	filtration unit	vessel
I-F-02	Gas evacuation to the outside in case of overpressure in the filtrate tank	Emergency valve left unconnected to release gas as fast as possible
I-F-03	Outlet of filtrate (drain) from filtrate tank (Interface CI/CII)	Multifix tube (EPTM) + mobile drain vessel
Gas Loop		
I-G-01	Evacuation of gas produced in bioreactor and measured in columns to the outside	Flexible tubing 6 mm and outlet to the outside
I-G-02	N_2 supply	N_2 bottle, tap, pressure regulator and flexible tubing 6 mm internal diameter
Cleaning		
I-C-01	Outlet (drain) of cleaning buffer	Multifix tube (EPTM) + mobile drain vessel
I-C-02	Gas evacuation to the outside in case of overpressure in the cleaning buffer	Emergency valve left unconnected to release gas as fast as possible
I-C-03	Water inlet in cleaning agent tank R-C-01	TriClamp bouch Clamp DN 20 (1) Butt weld to ½" female BSP (1) Gardena material to connect with water tap Hose multifix = 100 m 13 x 4 (12) Hose clip ABA-R SS304 19 x 28 (2)
Sterilization		
I-S-01	Outlet of steam condensate from the filtration unit	Flexible tubes and vessel

4.2.4 System closure

Gas tightness is necessary since the bioreactor must work anaerobically and the filtrate must be sterile. The bioreactor and the different associated tanks will be kept under a slight overpressure of 100 mbar. This is a double safety to prevent any entrance of oxygen or contaminants in case of underpressure. Mechanical safety overpressure valves will be foreseen.

4.2.5 Sterilisation, maintenance, cleaning and safety issues

A CIP (Cleaning In Place) and SIP (Sterilization In Place) installation will be provided, that will allow the automated cleansing of the whole system, including tanks and tubes, and the steam-sterilization of the membranes and the filtrate line in the Filtration Unit. Both will be realized by a system of automated 3 way-valves inserted in the installation.
A buffer tank will be used for the cleansing, where the cleaning agent will be mixed with water and circulated through the different units. Some actions such as filling the buffer tank with a cleaning agent will be performed manually. The buffer tank will also be used to bring the

membranes at the appropriate temperature in the appropriate period of time in order to perform a steam-sterilization without risk for the membranes.

4.2.6 Culture strategy

The process characteristics for the optimal performance of the anaerobic sludge have been described in TN80.16.

A back-up culture for CI will not be foreseen. If needed, inocula from the MELiSSA Pilot Plant could be used.
The biomass concentration will be maintained around 40 g/l in the bioreactor to optimize the efficiency of the process.

4.2.7 Calibration strategy

Only pH probes will be calibrated on a regular basis. Frequency and calibration procedures will be presented in the User manual.
The other sensors are calibrated initially following the supplier's instructions. Pressure and temperature sensors are usually initially calibrated and re-calibrated after long periods of time such as once every year or every 2 years.

4.3 System design

4.3.1 Reactor concept

The concept and design of the compartment I were defined. The pilot compartment can be divided in several sub-systems:
- Bioreactor itself; where the degradation is performed,
- Solid Loop, which handles the influent preparation and feeding, and the drain handling,
- Liquid Loop, which includes a filtration unit with on-line instrumentation that allows retention of the biomass,
- Gas Loop, which allows to sample the biogas and to regulate the pressure in the bioreactor.

The liquefying reactor will be operated at 55°C (thermophilic conditions) and at a pH lower than 6 to avoid methane production. It will be operated in anaerobic conditions. It will be fed on a semi-continuous basis with influent that will be mixed and stored at 6 °C in an influent tank.

The bioreactor will be coupled to an ultrafiltration unit. The task of the membrane is to separate the non-biodegradable organic matter from the produced VFA and ammonium. The membrane is a ceramic tubular membrane that is used in a redundant system of tangential filtration. The redundant system allows to automatically switch between 2 parallel membrane and continue the filtration in case of fouling or clogging. It is very important, for safety reasons and to avoid contamination in the next compartment, to retain the bacteria present in the bioreactor by the filtration unit. Therefore the tangential membrane is coupled to an

additional organic dead-end filter. Also, the filtration unit will be equipped with cleaning and steam-sterilization in place devices, to allow preventive cleaning and sterilization of the unit. The filtration process will occur continuously.

A Gas Loop will also be coupled to the bioreactor. Pressure decrease will be compensated by automated addition of N_2 gas. In case of pressure increase (biogas production), the gas produced will be measured and evacuated using the same concept as in the MELISSA CI. When the pressure increases in the bioreactor, the valve before the Gas Loop tank opens and the gas enters into the tank. At a certain pressure, the valve before the tank closes. The pressure and temperature inside the tank are measured and registered. Then the valve after the tank opens and the gas can be evacuated to the outside. The registration allows to determine the amount of gas present in the tank based on temperature and pressure measurement.

The control system for the bioreactor will be done at level 0, using a PLC. An automated detection of membrane clogging will be performed by the PLC based on pressure measurement. The automated switch to the other membrane will be stirred by the PLC in such a case.
Alarms will be programmed in case of disturbances or failures.

4.3.2 Material selection

- ✓ Due to its fragility and low pressure tolerance, glass is not recommended for the CI reactor construction, especially when cleaning and sterilization in place is required.
- ✓ PP can only be used if the reactor will not be steam sterilised. Chemical disinfection is possible with certain chemicals. Polymers are easy to glue and solder and are light in weight.
- ✓ The problem with any metal and any alloy is corrosion. However, with the selection of the proper steel type, this problem can be avoided. The advantages are a high temperature and pressure resistance, a long life time and the possibility to include industry-standard sensor fittings and interfaces.

Based on these findings, a reactor constructed in stainless steel is preferred. Since no elevated Cl^--concentrations are to be expected, stainless steel AISI-316 can be used. Corrosion due to the gas composition is also not expected due to the absence of O_2 in the headspace of the reactor. H_2S itself is not corrosive towards stainless steel AISI-316 without the presence of O_2. Abrasive and electropolishing can be applied as in pharmaceutical industry, in order to minimize process contamination.
More information on material selection can be found in the MELiSSA TN71.7 of the contract Engineering of the Waste Compartment.

4.3.3 Concept scheme

Figure 11 presents the concept of the BELISSIMA CI.

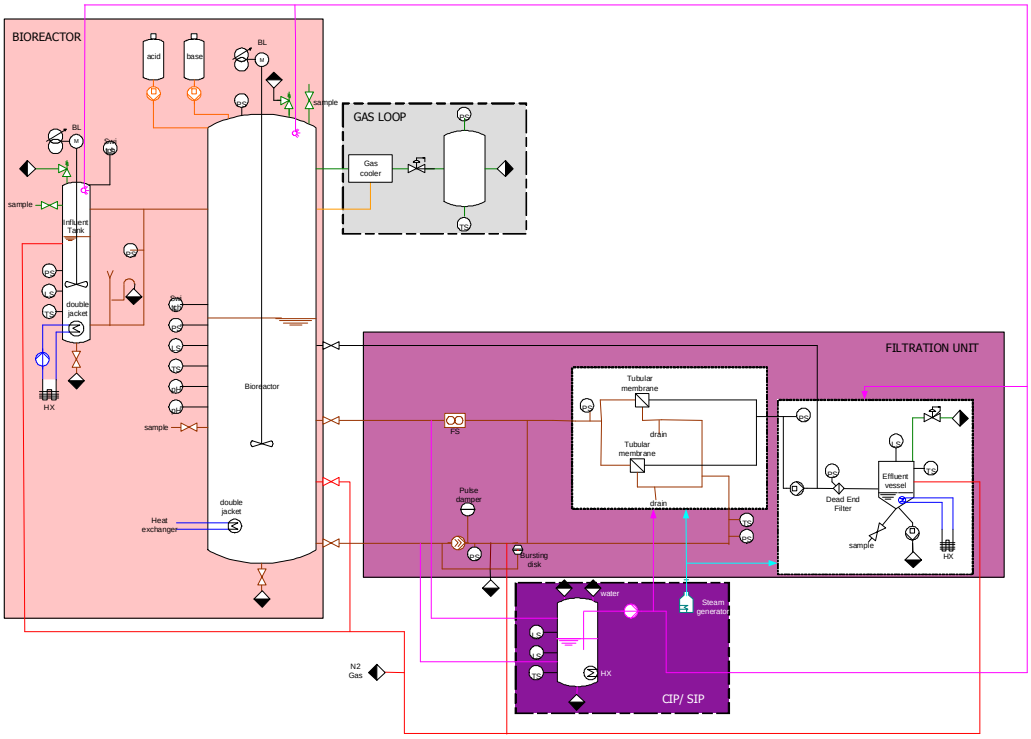

Figure 11: Simplified drawing of CI concept

5 Common design issues for compartments II-IVa

5.1 Temperature control bioreactor (and buffer tanks)

The influents and effluents of the bioreactors must be cooled to conserve the media. The desired temperature is 4°C and cooling down to this temperature demands a large cooling capacity. Maintaining the liquid at this temperature on the other hand is less demanding to the cooling system.

Compartments II and IVa are photobioreactors, illuminated by halogen lamps, which have a high heat dissipation. This results in a high demand for cooling capacity.
As cooling spirals may lead to biofilm formation and may reduce the illumination efficiency, double jackets are preferred. Both bioreactors and buffer tanks will be foreseen with a double jacket cooling system. The temperature in the reactor will be measured with a Pt100 sensor.

Temperature control can be performed in two ways:

1. *Central cold and warm process water circuit:* the cold and warm process water is being produced in a central place. The process water is circulated in a closed loop and has a constant temperature. For the tanks that need warm or cold process water a parallel branch is made. In fact, for each branch an individual temperature regulation should be provided. This temperature control is a function of the measured temperature in the medium to be controled. The disadvantage of such a system is the complexity. When the demand on a certain moment at a specific branch is high, the temperature control at the other branches might not be optimal. When different tanks (like the buffer tanks) or reactors must be kept at the same temperature of e.g. 4°C, a serial connection with one central temperature regulation is possible. This provides a more simple set-up of the system. The cooling capacity is determined by the ambient temperature.

2. *Individual temperature control:* every individual reactor or tank has its own production of process water. This can be meaningful when the ΔT between the reactors and/or tanks is too big to provide a reliable temperature control or when high demands towards the accuracy of the temperature regulation are required.

Suppliers:

- ✓ Central process water circuits: Lauda, Huber, Julabo, Haake-Neslab, …;
- ✓ Individual temperature control: Lauda, Huber, Julabo, Haake-Neslab, … .

Remarks and conclusions

All buffer tanks need to be kept at 4°C, so a sound decision could be the use of a central cold process water system. Dependent on the infrastructure and out of practical considerations, the choice must be made between one central system for the buffer tanks of all compartments, for a part of the BELISSIMA loop, or per compartment. Preference is given to the first option.

The bioreactors demand a continuous precise regulation of the temperature which requires individual temperature control. Therefore a temperature measurement in the medium of the bioreactor is necessary with feedback to an individual system of temperature control.
Since the bioreactors will presumably be made of glass, a pressure guarding system must be provided in the process water circulation system of the individual thermostat system for safety reasons.
Most commercial available bioreactors are provided with an individual temperature control system.

5.2 Gas (mixture) injection

Depending on the composition of the influent, carbon dioxide (CO_2) may have to be added to the reactor medium for compartment II. The addition can be performed from a gas cylinder with a certain concentration of CO_2 in a gas mixture with He. Also in compartment IVa, CO_2 may have to be be added to the air flow.
Via a pressure reducing valve, the addition of CO_2 can be performed in different ways:

1. *Manual controlled flow:* the desired gas flow is set by means of a needle valve and can be read out from a variable area flow meter. This is the simplest way to dose a certain amount of gas. The disadvantage is that with variations in pressure, no automatic correction of the flow is applied. This has to be taken into account when an accurate dosing is desired. The gas mixture must have a fixed composition. A change in the concentration causes a change in the flow reading because the density of the gas changes. The most simple systems do not allow flow registration.

   Suppliers: ABB, Brooks, Kytola Instruments, Gilmont Instruments, Platon, Vögtlin,…

2. *Control by a preset flow:* the difference with the possibility described above is that the flow is not only measured but also guarded and controlled by a proportional functioning magnetic valve. The measurement is performed by a thermal mass flow measurement. The desired flow is set manually. Deviations compared to the preset flow are corrected automatically by an internal controller which opens or closes the proportional functioning magnetic valve. Also this flow measurement is dependent on the density of the gas, which means that gas mixtures of a certain constant composition must be used. The signal from the thermal mass flow measurement can also be used for the registration of the flow and can be integrated in the global process of the compartment.

   Suppliers: MKS, Bronkhorst High-Tech, Brooks Instruments, Aalborg Instruments, Sierra Instrument,…

Remarks and conclusions

The first possibility is the most simple and is mostly satisfactory when no high demands are asked concerning measurement accuracy and when no registration is needed. For long term

experiments, requiring a constant accurate dosing of a gas mixture, possibility 2 is the best choice. The extra advantage is that the flow can be registered and integrated into the process resulting in a history file of the dosed quantity of gas. This is important to get mass balances with the BELISSIMA study.

5.3 Pumps for pH correction

The choice for a certain type of pump is determined by the reactor volume, the overpressure in the reactor,…

There are three types of pumps that could be used for pH correction purposes:

1. *Dosing by plunger membrane dosing pumps:* These pumps are applied frequently in industry and in wastewater treatment facilities to dose acids, alkali and reagents. They have a very wide flow range. The smallest types have a lowest adjustable flow of about 0,12 ml/h. They function at best at a constant set counterpressure. Mostly this pressure is 10 bar and is generated by a back-pressure valve. The pump head contains a suction valve, a delivery valve and a plunger with a membrane. The membrane is fixed to the plunger and functions as a separation membrane between plunger and the valve system. The functioning of the pump is electromagnetic and is obtained by a pulsating signal which moves the plunger back and forth. Variation of the flow is obtained by setting the stroke and the pulse-frequency. The choice of the material in which the pump housing and the valve system is made depends on the medium to be transported.

2. *Dosing by peristaltic pumps:* also these pumps have a wide range of application and are especially used in the fields of biotechnology, food industry, pharmacy and chemical industry. These pumps have a very wide flow range. The lowest adjustable flow is about 0,06 ml/h. The working principle of a peristaltic pump is simple and is based on the displacement of a fluid or product through a tubing which is alternately compressed and released. Therefore a flexible tube is positioned in a pumphead where at certain points it is completely pushed against a saddle by the rollers. As the rotor turns, the part of the tube under compression by the rollers closes, thus forcing the fluid to be pumped to move at constant volume through the tube without leakage. At the inlet side of the pumphead, as the tube opens, it creates an underpressure which induces new medium into the pumphead. The only part of the pump in contact with the fluid is the flexible tubing. The choice of the hose depends on the medium to be transported and the application.

3. *Dosing by gear pumps:* The pumping action of gear pumps is performed by two spur gears meshing together and revolving in opposite directions within a casing. The fields of application are in the food and the chemical industry for transportation of pure products with high viscosities. The pump housing has a suction and a delivery side. Power is applied to the shaft of one of the gears, which transmits power to the driven gear through their meshing teeth. Liquid is being sucked at the inlet and fills the empty space between the successive gear teeth and the case and must follow along with the teeth as they revolve. When the gear teeth mesh with the teeth of the other gear, the space between the teeth is

reduced and the entrapped liquid is forced out of the pump discharge pipe. Most gear pumps are fabricated with a magnetic drive mechanism which makes them seal-less. The minimum flow ranges are around 48 ml/h. The choice of the material of the pump house depends on the products to be pumped.

Suppliers:
- ✓ Plunger dosing pumps: Prominent, Iwaki, Milton Roy, …;
- ✓ Peristaltic pumps: Ismatec, Masterflex, Verder, Watson Marlow, …;
- ✓ Gear pumps: Micropump, Iwaki, Tuthill, Kracht, …

Remarks and conclusion:
Commercial bioreactors have the pH-control together with the dosing pumps integrated into the system. The pumps applied in these systems are peristaltic pumps.
In the other case, the three types of pumps are equal in functionality, but for smaller reactors and with respect to maintenance and simplicity of action, the peristaltic pumps are the best choice.

5.4 Illumination and control

As stated in the requirements, illumination of compartment II and IVa has to be done with halogen lamps. To be able to vary the illumination between 0 and 300 W/m², the light intensity will be varied through adjustable power. The biological process of CII requires furthermore a continuous alteration between light and dark zones.

There are two different methods for the control of the illumination:

1. *The manual adjustment of the electrical potential:* being the most simple construction. By means of a potential variation and an independent light measurement the required electrical potential setpoint can be entered. This is done once for a certain working time. The light intensity however will decrease in time due to wear of the halogen lamps. The technical characteristics of the lamps will determine the achievable working time of the halogen lamps.
2. *Control of the illumination by measuring the light intensity in the photobioreactor:* this option is comparable to the previous one. However, the adjustment of the light intensity is automated by means of an intensity sensor somewhere in the reactor. This solution offers the advantages that wear of the halogen lamps influences less the light intensity.

Remarks and conclusion
Illumination by means of halogen lamps is required within MELiSSA-projects. Halogen lamps have however an important disadvantage: they need high current intensities and produce as a consequence heat. LEDs and fluorescent lamps imply less complex constructions and do not produce heat. Due to the heat production of the halogen lamps ventilation around the bioreactor will be needed.
Of the two methods mentioned for control of the illumination, the first one is selected because of its simplicity. Furthermore, the experience of UBP learns that the wear on the lamps is

negligible and that replacing the lamps after e.g. 1 year would be sufficient to maintain the required light intensity.

5.5 Overpressure control of bioreactor and buffer tanks

The reactor and buffer tanks are kept at a small overpressure of 100 mbar to avoid contamination of the media from the ambient atmosphere. The propellant used can be either pressurized argon or helium. Argon has the disadvantage that it is highly soluble in water and therefore, more gas will be needed to maintain a certain overpressure. Helium is more expensive , but has a lower solubility.

Different options are available to have a system at overpressure:

1. *Manual control valve + pressure indicator + flow indicator:* a slight overpressure can be created by means of a constant gas flow on top of the liquid phase. By means of a regulation valve a slight overpressure can be maintained that is indicated on a pressure indicator. This corresponds to a certain gas flow.

Suppliers:
- ✓ Pressure indicator/trancducer: Endress + Hauser, Kulite, Vega, Keller, Honeywell, Danfoss, Fischer Mess- und Regeltechnik, …;
- ✓ Manuel control valve: Parker, Swagelok, …
- ✓ Flow indicator: ABB, Brooks, Kytola Instruments, Gilmont Instruments, Platon, Vögtlin, …

2. *Pressure switch + electromagnetic valve (on/off):* an electromagnetic valve is controlled and opened when a certain setpoint has been reached and is closed when at a maximum set pressure. This is the most simple and cheapest way to keep a system at a certain overpressure. The drawback, however, with a system that switches on/off between two setpoints is that there are fluctuations in the system which might have a negative influence on the working conditions. In many cases this way of control can be applied e.g. in storage tanks not directly in connection with other systems sensible to pressure variations.

Suppliers:
- ✓ Pressure switches: Endress + Hauser, Kulite, Vega, Keller, Honeywell, Danfoss, Fischer Mess- und Regeltechnik, …;
- ✓ Electromagnetic valves: Bürkert, Asco, Parker, Danfoss, …

3. *Pressure measurement (transmitter) + electromagnetic valve (proportional) + control loop:* This combination is more complex. A continuous control of a preset pressure is realized which results in a nearly constant pressure. The pressure sensor provides a signal (according to a certain pressure) towards a controller. Dependent on the setpoint, the controller calculates a steering signal to a connected proportional acting electromagnetic valve. Depending on the deviation of the actual pressure in relation to the preset pressure, the control valve is opened at certain position. This results in a constant pressure

regulation, depending on the set control parameters (proportional band). This combination can be composed by individual components, but is also available as one unit. In such case, a pressure regulator is coupled to a thermal mass flow meter. The function is very stable and accurate.

Suppliers:

- ✓ Pressure sensors: Endress + Hauser, Kulite, Vega, Keller, Honeywell, Danfoss, Fischer Mess- und Regeltechnik, …;
- ✓ Proportional electromagnetic valves: MKS, Aalborg Instruments, Bronkhorst Hi-Tec, Brooks Instruments, Asco, ...
- ✓ Combined systems: MKS, Bronkhorst High-Tech, Brooks Instruments, Aalborg Instruments, Sierra Instruments, …

Remarks and conclusions:
For the commercially available bioreactors all options can be integrated in the concept. The third option is the most accurate and as a result preferred for the BELISSIMA loop.

5.6 Feed to the bioreactor

The flow towards the bioreactors is rather small, which limits the options for transporting the feed. The maximum adjustable flow is estimated around 12 l/d.
There are two ways to feed the bioreactors:

- ✓ a peristaltic pump;
- ✓ transport by means of an adjustable pressure.

For reasons of control and setting up a mass balance an accurate flow measurement or measurement of the dosed quantity is recommended.

The different possibilities are:

1. *Peristaltic pump + weighing of the influent tank:* An accurately adjustable and controllable peristaltic pump provides the feed towards the bioreactor. A fixed or intermittent (puls-pause) flow is set. The consumption is registered in time by means of an analytical balance. The disadvantage of this system is that the real flow can deviate from the set flow by wear or by inexactness of the tube. This problem can be solved when the registration of the weight is fed back towards the pump speed. The registration of the consumption of the influent can show inaccuracies when e.g. the tank on the balance is stirred or when connections are made to the influent tank (like in this case). An important factor to avoid these problems is the way the buffer tank is constructed and the manner of the integration of the weight measurement.

2. *Peristaltic pump + mass flow measurement + weighing of the influent tank:* This set-up differs from the previous one by the integration of a flow measurement. Thus, the flow is registered in time from which the consumption is calculated. Weighing the influent tank is not necessary, but can serve as control of the flow measurement. For an accurate mass flow

measurement of small quantities, only two measurement principles apply: “thermal” and “Coriolis” mass flow measurement. Thermal mass flow measurement for liquids can only be applied when the physical parameters heat capacity and thermal conductivity are invariable. This principle is not applicable since these characteristics of the influent are variable for BELISSIMA. The “Coriolis” principle is insensitive to these characteristics and is furthermore a very exact measurement, so the only valuable alternative to the analytical balance.

3. *Peristaltic pump + mass flow control + weighing of the influent tank:* This set-up is almost identical to the one mentioned before, except for an integrated flow control. The output signal of the mass flow measurement is further used for mass flow control. Therefore a controllable peristaltic pump is required. The above mentioned remarks apply here as well.

4. *Liquid transport by means of overpressure + mass flow measurement + weighing of the influent tank:* The influent tank is continuously kept at slight overpressure to avoid contamination from the ambient atmosphere. This overpressure can also be used to feed the bioreactor. Hereby an accurate overpressure regulation is required, as described in 5.5, in order to create a constant overpressure in the influent tank. In this set-up only a mass flow measurement is provided, whereas the flow is registered in time to obtain the consumption of the feed. The feed of the bioreactor is determined by the exactness of the overpressure regulation of the influent tank and the bioreactor. Possible variations in pressure in both tanks will have an influence on the desired flow.

5. *Liquid transport by means of overpressure + mass flow control + weighing of the influent tank:* this is a set-up that resembles the previous principle. The difference is that an independent mass flow regulation is applied to avoid the influence of pressure fluctuations on the flow. This results in a more stable feed. The regulation is performed by controlling a proportional acting electro-magnetic valve designed for liquids. To avoid feasible obstructions or irregularities a filter can be placed before the mass flow controller.

Suppliers:

- ✓ Peristaltic pumps for small flow: Ismatec, Masterflex, Gilson, Alitea, Pharmacia Biotech, Watson-Marlow…;
- ✓ Analytical balances: Mettler Toledo, Sartorius, …;
- ✓ Mass flow measurement (control) for liquids: Bronkhorst High-Tech, Brooks Instruments, …

Remarks and conclusions

Peristaltic feeding pumps, with a pulse-pause control, are standard on commercially available bioreactors. Within BELISSIMA the feeding flow rates will be rather low. In addition accurate mass balances are required. Option 1 is the most simple solution and provides sufficient accuracy. There must be a feed back from the weight measurement towards the pump to correct for deviations.

Options 3, 4 and 5 are more complex. Option 1 and 5 imply that the influent buffer cannot be seen as a separate interface. It will be part of the bioreactor and its control.

A pressure driven feeding system requires a stable process operation. In case of connection of different compartments in a future stage, it will be difficult to obtain a stable overall process with its different pressures in the successive compartments and buffering tanks.
All suggested options can be integrated into the commercial bioreactors upon request.

As the flow rates within the BELISSIMA study are rather low, all suppliers suggest to work with a gravimetrical controlled feeding of the bioreactor. The balance controls the pump speed.

5.7 Effluent of the bioreactor

The influent is fed at a fixed flow rate to the bioreactor, depending on the composition and active volume of the bioreactor. The effluent should be evacuated at a comparable flow rate in order to maintain a constant liquid level in the reactor.

The effluent can be transported out of the reactor in different ways:

1. *Pumping the effluent at an identical flow rate*: when the influent is pumped by means of a peristaltic pump, the same pump driver or an identical pump and tubing could be used for the evacuation of the effluent. This approach however implies some risks. Due to wear of the tubing or a non identical pump speed the reactor can be drained or run over.
2. *Pumping at a fixed liquid level in the reactor:* This would be the most simple design to keep the liquid level at a constant value. The suction is done at an adjustable level in the reactor. The pumping speed is set slightly higher than the speed of the inlet pump. An important disadvantage could be that gases are evacuated as well out of the reactor through the effluent piping. These gases can be produced in the bioreactor or can be entered by the overpressure regulation system. A chemostate tube can offer a solution.
3. *Peristaltic pump + weighing of the effluent tank + level masurement*: the influent flow should be identical to the effluent flow. However, as mentioned under option 1, separate influent and effluent pumps will never yield identical flows, because of wear of tubings or small differences in pump speed. To avoid drainage or overflow, a control system can be included, based on weighing the effluent tank or continuous level measurement.
4. *Peristaltic pump + weighing or level measurement of reactor vessel:* the working volume of the reactor corresponds to a specific weight. Hence, once the desired weight or corresponding liquid level is exceeded, the effluent pump needs to be activated. The pumping itself can be performed either at a fixed flow or through proportional control. A level measurement is an alternative to weighing, but is less accurate since it can be confounded by biofilm or foam formation.

Suppliers:

- ✓ peristaltic pumps for small flow rates: Ismatec, Masterflex, Gilson, Alitea, Pharmacia Biotec, …

Remarks and conclusions:
Option 2 is implemented in most commercial hardware.
The most accurate option to close mass balances is option 4. Control through weighing is preferred to level measurement because it is not affected by biofilm or foam formation.

5.8 Influent and effluent buffer tanks (interfaces)

The flow through the loop will be ~12 l/day or 8,33 ml/minute. The buffering volume should be sufficiently large to cover at least 3 days. This means that the volumes of the buffer tanks should be around 40 liters. As stated in the requirements, the illumination of the tanks should be minimal and temperature must be reduced to 4°C.

There are three options for buffering the influent and effluent:

1. *Sterile synthetic bags:* these bags are commercially available in different volumes, up to 1000 liters. They can only be used once. For long-time experiments this would imply an important operation cost. The cooling of this type of bags can be done in a cold storage room. This would have the advantage that no mixing device is required. On the other hand, the disadvantages are that a gravimetric follow-up is not possible and an overpressure, as required, is difficult to realise.
   The sterile influent should be fed to the buffer tank by means of a peristaltic pump. In the same way, feeding from the cold storage room towards the bioreactor must be foreseen. When using synthetic buffer tanks, there will be a risk of release of certain chemicals. Within the BELISSIMA study, this has to be avoided.

2. *Autoclavable synthetic or SS tanks*: this is comparable to option 1. The difference lies in the fact that the tanks are reusable. Sterilisation must be done in an autoclave. The tanks can be preserved in a cold storage room. Comparable to option 1 no mixing device will be needed. On the other hand, a gravimetric follow-up of the consumption will be possible. Only minor release of chemicals out of the material is to be expected. Traces of Cr, Ni and Fe however could be found in the solution. An important disadvantage of this option is that large volumes in stainless steel become very heavy and difficult to operate.

3. *In-situ sterilisable SS tanks:* this will be the most complex solution, as the buffer tanks will have to remain in a fixed position. The cooling of the solution will be done by means of a double jacket or spiral fed with cooling water. The buffer tanks are air tight to avoid release of vapours. For optimal cooling a mixing device is recommended. As the tanks are fixed, a separate autoclave will be needed to sterilise a different SS receptacle containing the influent. For the transport of the solution to the buffering tank, a pump or pressure driven system could be used. The influent should be filtered before feeding the buffering tank. The feeding towards the bioreactor requires then an in-situ sterilisable SS tubing. During cleaning phases the influent buffer could be used as a storage vessel for the cleaning products. The liquids are pumped by a dedicated pump through the tubings and circulated during an adjustable time.

<u>Suppliers:</u>

- ✓ Sterile synthetic bags: Sartorius, …;
- ✓ Autoclavable synthetic or SS vessels: suppliers of laboratory equipment, SS constructors, …;

✓ In-situ sterilisable SS vessels: Sartorius, Bioengineering, SS constructors, ….

Remarks and conclusions:
Option 3 is preferred since manual operations are minimized, compared to autoclavable vessels. In-situ sterilisation reduces the risk of contamination of the system. Finally, stainless steel will leach less additives compared to polymer tanks and illumination of the collected suspension does not take place.

5.9 Solid-liquid separation

With an effluent production of about 12 l per day, between 20 and 30 l of cell suspension will be collected in the effluent tanks over a couple of days. This is the volume which has to be treated in the solid-liquid separation system. As described in TN72.2.2, centrifugation or membrane filtration are the selected technologies for liquid-solid separation in compartments II and IVa. The output of CIII also requires cell removal through centrifugation or filtration before the clear supernatant is sent to CIVa, but here the amount of cells to be removed will be lower.
For preliminary lab-scale tests on the connection of CII, CIII and CIVa, TN43.8 describes the use of a batch centrifuge. This technique has also been used until now in the MELiSSA Pilot Plant. Centrifuging has the disadvantage that the supernatant is not completely free of cells and that cells may be damaged or altered. Within a closed Belissima loop a dead-end filtration will be preferable to achieve optimal cell removal and maximal filtrate.

5.9.1 Centrifugal separation

Centrifugation is a technique which uses high energy to subject e.g. liquid-solid suspensions to sedimentation or filtration with the aim to separate the 2 phases. The acceleration is obtained through fast rotation of a basket (perforated or not), around an axis. The ratio of the acceleration ($\omega^2 r$) to gravity ($\omega^2 r/g$) is a measure for the force of separation. This amounts to several thousands of g for some industrial centrifuges.

For application on the CII, CIII or CIVa effluent, there are 3 possible scenario's:
→ Laboratory batch centrifuges;
→ Laboratory continuous centrifuges;
→ Industrial batch centrifuges.

1. Laboratory batch centrifuges (see Figure 12): these are usually classical table centrifuges which can be used for various applications. The volume which can be treated is typically limited to 3 – 4 l depending on the supplier. Some suppliers have models which can treat up to 8 – 12 l.

Figure 12: Benchtop cooled laboratory centrifuge 6K15 (Sartorius)

Laboratory batch centrifuges exist in different forms depending on the application. They may vary in the type of rotor for specific volumes and containers and in the possibilities for acceleration and braking. They are mainly used in clinical applications, food and microbiology and to a lesser extent in industry and environmental applications. They are interesting for small volumes. When relatively larger volumes need to be treated, the number of manipulations and concomitantly the risk of contamination will increase. For the latter applications, the centrifuge will have to be placed in a room with a controlled atmosphere. Table 15 shows some technical specifications for one type of lab centrifuge.

Table 15: Technical specifications for lab centrifuge (Sartorius Serie 6K15).

Type	Sartorius 6K15
Maximal capacity (l)	3
Rotations per min	15000
maximal g–value for 3 l	5300

Suppliers: Sartorius, Beckman Coulter, Eppendorf, Jouan (Thermo Electron), Heaeus, …
Indicative price for a cooled centrifuge for a volume of 4 x 750 ml: >10000 €

2. Laboratory continuous centrifuges: potential suppliers and possibilities are limited. In pharmaceutical and clinical applications the CEPA centrifuge is well known (see Figure 13 for a picture of type LE and Table 16 for some technical specifications).

Figure 13: Continuous lab centrifuge CEPA Series LE

Table 16: Technical specifications CEPA Series LE/GLE

Type	LE/GLE
Capacity (l/h)	30
rotations per min	15000 - 40000
maximal g–value.	40000
Internal volume (l)	0,25

The GLE type is steam sterilizable (see Figure 14). This does however not guarantee that it can be operated under sterile conditions since the medium is still in contact with the surrounding air. To maintain sterility, the centrifuge must be placed in a controlled atmosphere.

Figure 14: Continuous lab centrifuge CEPA Serie GLE

Supplier: Carl Padberg

Indicative prices:

Type	€
LE	27600
GLE	35700

An alternative to the previous techniques is based on separation through a liquid/liquid centrifugal seperator/extractor. This is a patented system developed by the company CINC in Germany. Figure 15 shows type V2.

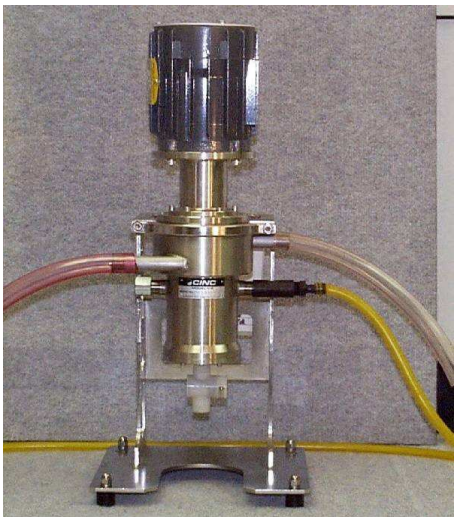

Figure 15: CINC ® V2 Centrifugal separator/extractor

The principle of operation is given in Figure 16. The liquid enters sideways and subsequently gets into the lowest rotor part through a mixing zone. In the space between rotor and stator the liquid is separated through centrifugal forces into a heavier (blue) and a lighter phase (yellow). The heavier phase is driven to the outer zone. Both fractions are discharged separately at the top of the apparatus.

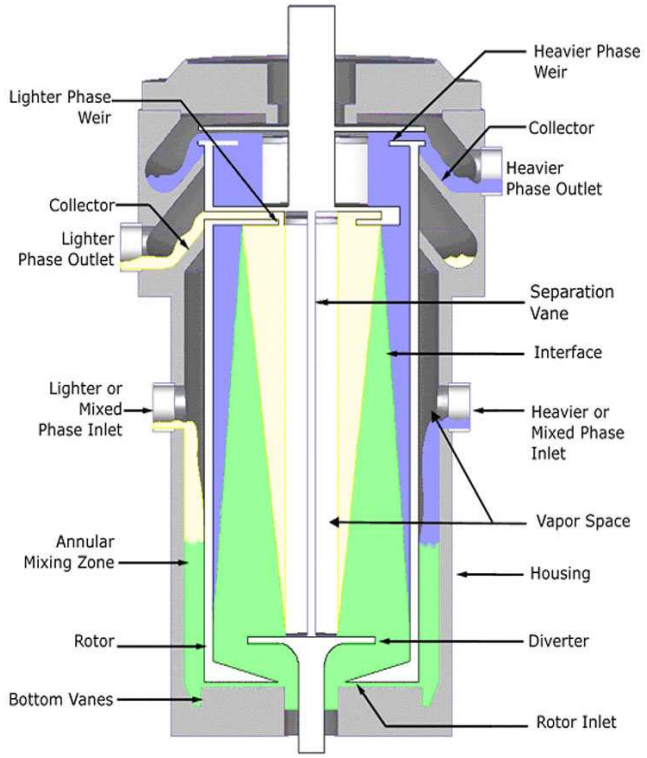

Figure 16: Principle of operation of the V2 seperator

The system is autoclavable and CIP cleaning is possible. The apparatus is mainly used in the pharmaceutical, chemical, biotechnological, food, oil and metal industry but also in environmental applications.

Some technical specifications are given in Table 17.

Table 17: Technical specifications CINC ® V2 Centrifugal separator/extractor

Type	V 20
Capacity (l/h)	120
Rotations per min	1000 - 3000
g–value	5000 - 80000
Internal volume (l)	not given

Supplier: CINC Deutschland GmbH

Indicative price: 9000 €

3. Industrial batch centrifuges (see Figure 17 and Table 18): these drum centrifuges distinguish themselves from the previous ones by the fact that they do not operate in continuous mode but can handle large volumes at once. Depending on the type,

volumes of a few liters up to 500 l can be treated. These centrifuges contain a perforated drum which has to be specified as a function of the medium to be treated. They are available in sterilisable form.

Figure 17: Industrial batch centrifuge CEPA Series TZ

Table 18: Some technical specifications of CEPA centrifuge type TZ4

Type	TZ4
Capacity (l)	20
Rotations per min	1600 - 2100
g-value	-

Suppliers: Carl Padberg, Rouselet Robatel, Westfalia separators, Alfa Laval, …

Indicative price: strongly dependent on type, capacity and design

5.9.2 Separation through membrane filtration

The term membrane filtration stands for diverse, highly different separation processes. They have in common that a semi-permeable membrane is responsible for the actual separating effect. The driving force for permeation through the membranes is a pressure difference over the membrane, a concentration difference or differences in electric potential. The membrane functions as a selective barrier, through which some compounds permeate whereas others are retained.

Two main factors determine the membrane filtration process, the selectivity and the productivity. The selectivity is expressed through the parameter retention or separation factor, productivity through the parameter flux. Both are membrane-dependent.

For the current applications, microorganisms need to be separated from the spent medium. Therefore, the processes of interest are micro- and ultrafiltration. In this case, separation is based on a physical process where the membrane pore size determines the degree of removal for suspended solids, turbidity and micro-organisms. Compounds larger than the pore size are removed. Smaller ones will pass through the membrane, although the occurrence of a gel/fouling layer may remove them to some extent. For microfiltration membranes, the pore size is typically between 0,1 – 10 µm, for ultrafiltration between 0,001 – 0,1 µm.

Membrane filtration systems can be operated in dead-end or cross-flow. In the former case, all feed water is forced through the membrane. Due to accumulation of components at the membrane surface, filtration resistance will increase and membrane cleaning is necessary. In cross-flow or tangential filtration, the feed flow is parallel to the membrane. A small fraction is produced as permeate, whereas the largest fraction leaves the module. The flow rate along the membrane is kept sufficiently high to reduce the formation of a fouling layer. Cross-flow operation results in stable fluxes. Here as well, membrane cleaning will be needed at certain frequencies.

Continuous flow filtration of bioreactor effluents can be achieved through depth filtration (dead-end) or cross-flow filtration.

1. Depth filtration is a non-stationary process in which the following phenomena take place:
   - → Sieving;
   - → Sedimentation;
   - → Interception;
   - → Flocculation.

   In depth filtration the concentration of the solids in the liquid and on the filter medium changes, each as a function of time.

   Depth filtration is a very simple technique with a minimal loss of medium. Various concepts are available. Well-known is the use of filtering cloth. An alternative is the closed filter cartridge. The module can contain different filter media and exists for various capacities. Figure 18 and Figure 19 give an overview of designs. The PALL Supradisc™ module consists of disk membranes with one layer of fine pores and one layer of larger pores. This increases the solid loading capacity of the membranes.

Figure 18: PALL Supracap™ 60, Supradisc™ and Supradisc™ HP depth filtration modules

Figure 19: Millipore Millistak minicapsule, Millistak HC POD, Millistak HC depth filters

Figure 20 shows the operating principle of a depth filtration module. The feed is pumped to the outside of the module, passes through the filter medium and is collected and removed centrally.

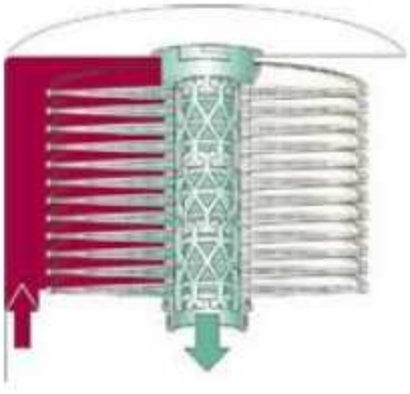

Figure 20: Principle of operation for PALL Supradisc™

Suppliers: Pall, Millipore, Whatman

2. Cross-flow filtration: various commercial systems are available targeting biomass harvesting. The main disadvantage of these systems is their “hold up” volume. For our application this should be as small as possible to minimize medium losses. Some typical commercial cross-flow membrane filtration system are shown in Figure 21 and Figure 22).

In a cross flow operation mode, fouling of the membrane can be reduced. Contrary to centrifugation and dead-end filtration, solids will only be harvested as a slurry rather than a paste. This is a very important disadvantage of cross-flow membrane filtration when closed loop operation is aimed for. On the other hand, an advantage of the membrane technology is that the permeate is cell free.

Figure 21: Whatman ULTRAN® - Maxiflex

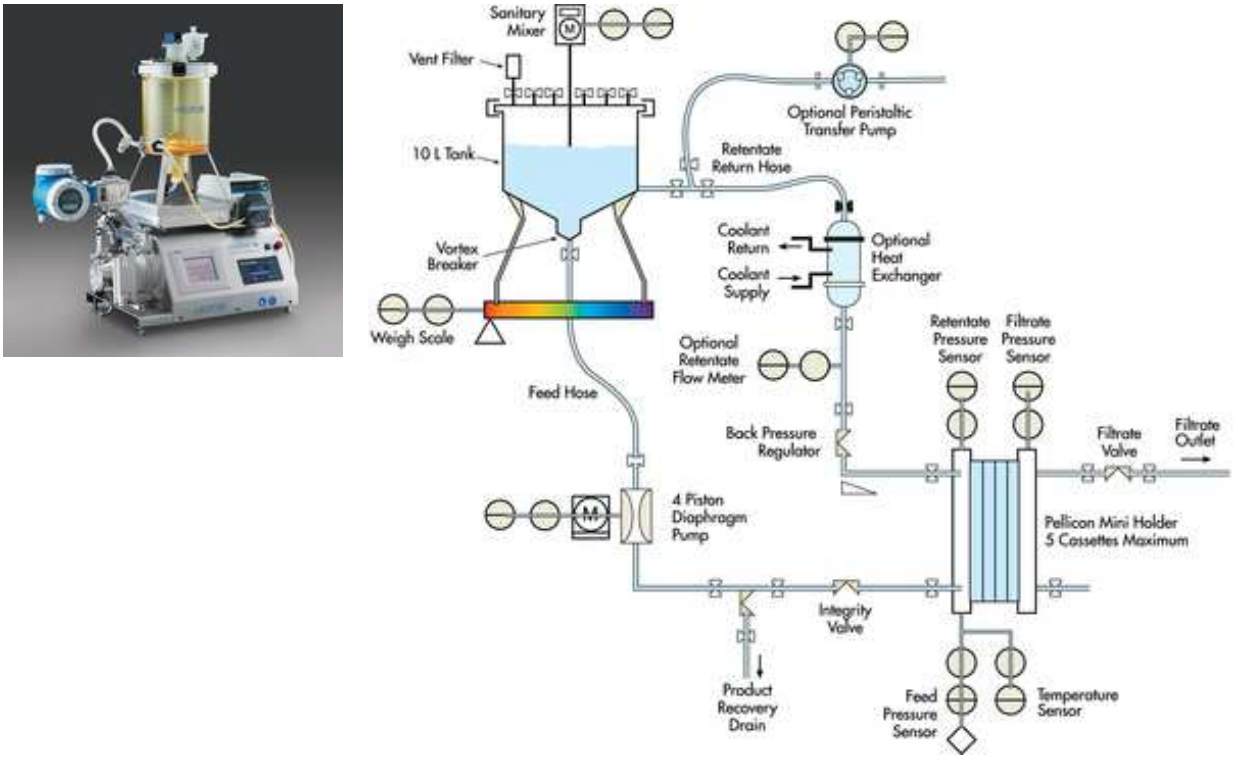

Figure 22: Millipore Cogent™ Tangential flow filtration, “Hold-up” volume < 10 ml (without cassette)

Suppliers: Pall, Millipore, Whatman; Sartorius, …

Remarks and conclusions
Currently, a selection of the best solid-liquid separation system cannot be made. Once the cell suspension to be treated is available, test runs will be performed with centrifugation and membrane filtration systems. The evaluation criteria will be

- Efficiency of liquid-solid separation (should be maximal)
- Risk of breakthrough of cells (should be minimal)
- Recovery of liquid phase (maximal liquid loop closure is aimed for)
- Consumables (e.g. cleaning agents in continuous filtration processes, should be minimal)
- Manpower/cost.

As opposed to dead-end filtration, crossflow filtration leads to significant water losses in the concentrated cell suspension. This is not optimal in a closed loop system. However, at the high cell concentrations that need to be harvested, it is preferable.

5.10 Process automation

Commercial bioreactors are autonomously working devices with their dedicated control programs. However, the program should be flexible in a way that changes can be made to the process control. External communication by means of analog or digital in- and outputs must be foreseen. It must be possible to make changes in the process control independently from the supplier. External devices and/or instruments should be easy to connect and integrate in the control system. It must be possible to generate actions related to external signals, e.g. a full influent tank of CIII should result in an alarm and stand-by of CII.

Suppliers of commercial hardware all have their own type of control system for the biological processes. Two major different solutions are available:

1. *PC-dependent process control*: the software is present on a PC and controls the process of the bioreactor. The control module of the bioreactor takes the actions. The digital communication in-between the software and the control module is done by means of a chosen ‘BUS’ protocol (Figure 23). The operation and the configuration of the process is done through the PC.

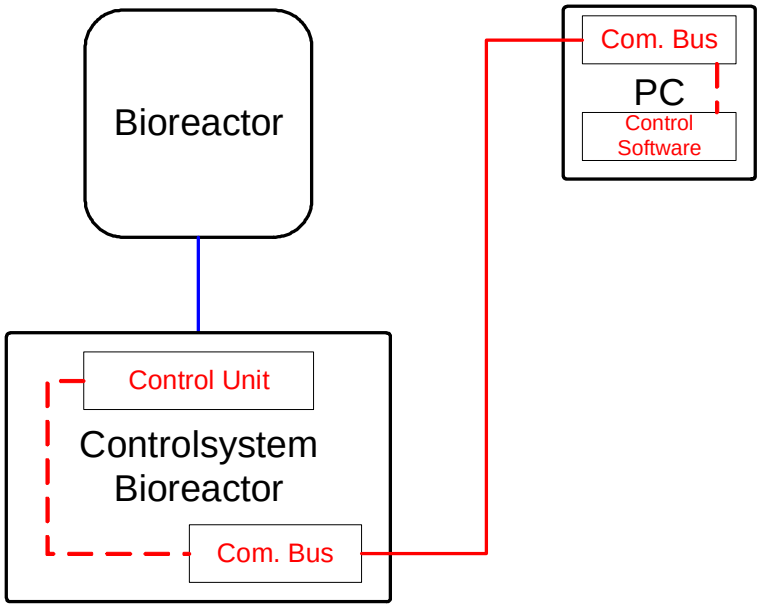

Figure 23: PC dependent process control

2. *Local process control, PC independent*: The final result of this control system is identical to option 1. The important difference with the previous solution is that the software and the control module are integrated into one. The operation and configuration of the control in this case is done through an operation panel (display). The principles are presented in Figure 24. A digital communication is provided for visualisation on a PC and data logging.

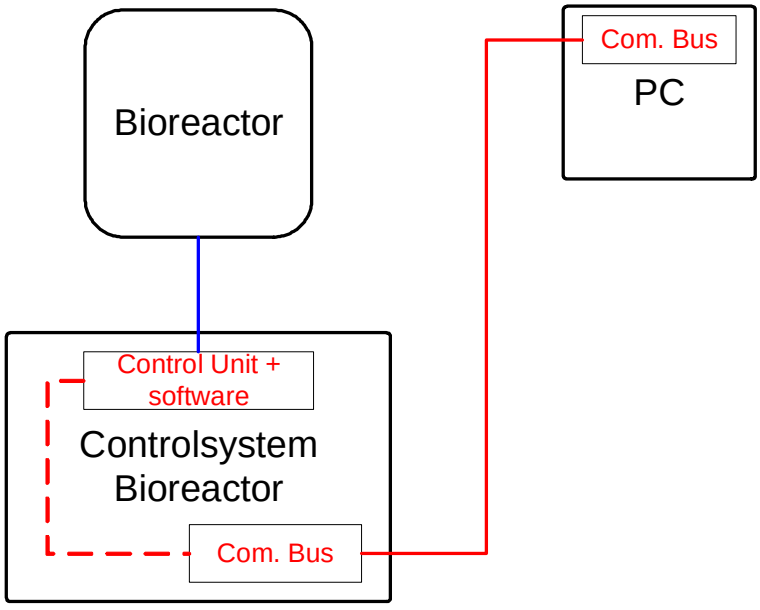

Figure 24: Local proces control

In order to guarantee a flexible possible future extension of the possibilities of the bioreactor the following list of requirements is given:

- ✓ analog outputs for every measurement (AO, e.g. 4-20 mA):
- ✓ analog inputs for external devices and instruments (AI, e.g. 4-20 mA);
- ✓ tensionless outputs (DO) that can be configurated for particular events (alarm generation);
- ✓ tensionless inputs (DI) that can be configurated for specific external events;
- ✓ digital communication bus for the transfer, visualisation and data logging;
- ✓ digital communication 'bus' for external devices, as for instance a Schneider PLC.
- ✓ software that can be easily configurated by own staff;
- ✓ easy integration and configuration of external devices and instruments into the process.

Figure 25 illustrates how additional devices, instruments or control units (PLC) can be integrated easily in the process.

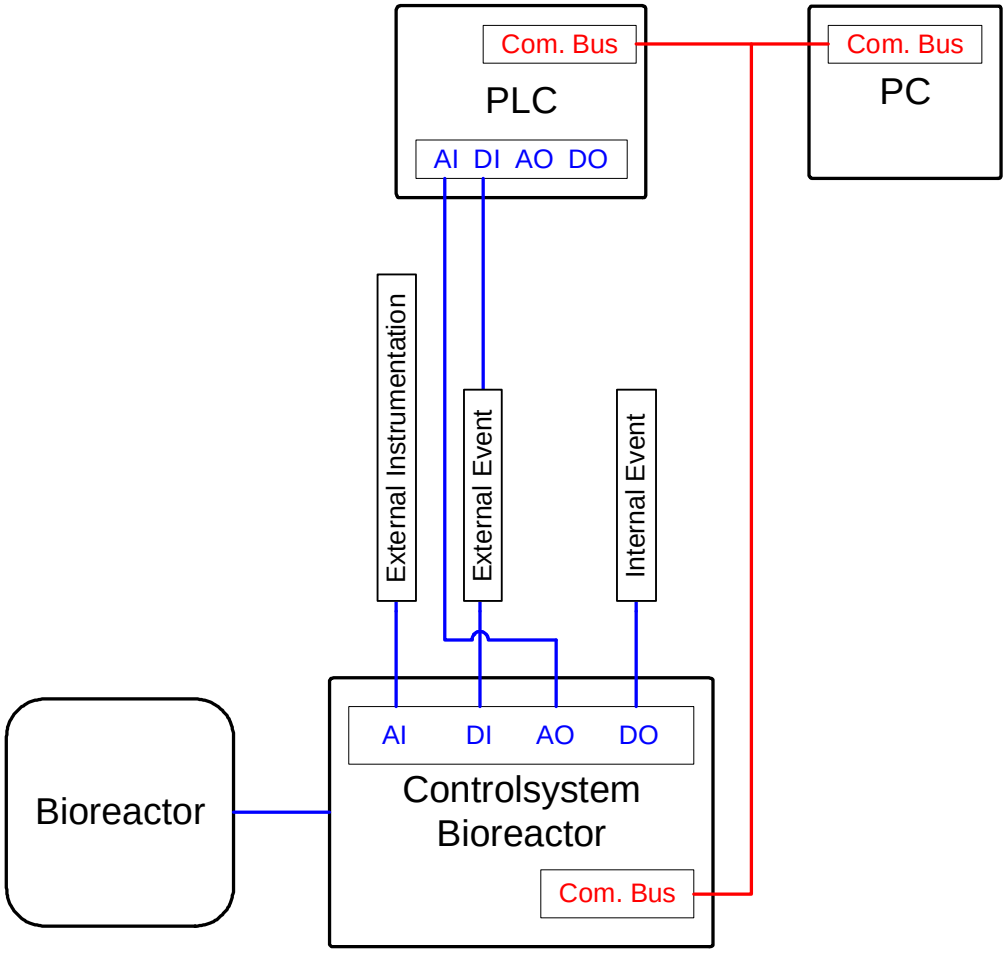

Figure 25: Simplified drawing of the requirements concerning communication

Suppliers:

- ✓ Controlsystem bioreactor: Sartorius, Bioengineering.
- ✓ PLC: Schneider

Remarks and conclusions

The local process controller of the bioreactor is an autonomous unit that offers sufficient flexibility. The configuration of the process must be adjustable so that external instruments and devices can be easily integrated. It should have as well the possibility to communicate with external devices for data logging, visualisation and control, such as a PC or PLC. Most commercial systems work in this way. Their control system will be tailor-made to the process and specific requirements. The systems might be more compact, reliable and cost effective than self-made systems composed of external transmitters, PLC and other measuring and control devices. Moreover, configuration and programming of commercial systems make use of the company's long-term experience of biotechnological processes, existing libraries of algorithms and programming software which has already been validated on various applications. For self-made systems, these aspects would have to be subcontracted. The various supplying alternatives will be further addressed in TN80.24.

6 Compartment II

6.1 Summary of design requirements

The design requirements for this mesophilic photoheterotrophic reactor were described in detail in TN80.16. Table 19 summarizes the technical requirements, related to the reactor itself.

Table 19: Summary of reactor requirements

	Detail	Requirement	Comment
Operation	Sterility	yes	
	Gas closure	yes	
	Pressure	100±20 mbar	slight overpressure
	pH-correction	yes	
Reactor	Shape	cilindrical	for optimal mixing and illumination
	Illumination	0 - 300 W/m² variable	light/dark zones (TN 49.2): - cycling between light and dark zones is required; - non illuminated headspace to be minimized.
	Stirring	good radial homogeneity: 300 - 400 rpm	mechanical mixing (TN 47.1): proven that less biofilm is formed compared to air-lift system
	Cooling	yes	due to the irradiation of the reactor cooling is required

6.2 Process characteristics

6.2.1 Sizing

The sizing of the overall BELISSIMA loop is based on the fixed working volume of this compartment. As a complete surface illumination is aimed for, a glass autoclavable bioreactor was selected. The maximal working volume of this type of bioreactors is around 10 liters. The design of the BELISSIMA loop is detailed in paragraph 2.2 and starts from a CII working volume of 10 l.

6.2.2 Functioning mode

The reactor will be operated in continuous mode. The feeding of the reactor will be done at a fixed flow rate, based on the reactor working volume and design dilution rate. The effluent flow rate will be determined by weighing of the reactor vessel.

6.2.3 Interfaces

The photobioreactor CII will have its own dedicated inlet and outlet storage. Both tanks will be provided with a mixing device. Especially the effluent vessel, containing a suspension of bacteria, should be homogeneous to guarantee optimal sampling. Furthermore, the buffering vessels will be cooled through a centralised cooling system. The volume will be sufficiently large to cover 3 days of operation and should therefore be in the order of 40 l.

Details on solid-liquid separation are given in 5.9. Whether centrifugation or membrane filtration will be selected as solid-liquid separation technique, will depend on test runs to be performed once compartment II is running and typical cell suspension is available. In addition, the outcomes of an ongoing study on the evaluation of membrane filtration techniques for *R. rubrum* harvesting (contract 20327/06/NL/PA) can then be taken into account.

The flow-rate of the off-gas of the reactor will be monitored. The composition will be determined off-line (see also 9.3).

Table 20: General overview of the interfaces of CII

Interface (tag P&ID)	Description	Needed equipment
Influent tank		
	Gas evacuation to the outside in case of overpressure in the influent tank	Stainless steel mechanical safety Overpressure valve
	Slight overpressure with He	Overpressure regulation and stainless steel valve
	Inlet of fresh influent	Stainless steel valve and piping
	Outlet of influent (drain)	Stainless steel valve and piping
	Cooling	external cool process water circuit + Temperature control + circulation pump
	Gravimetric determination tank content	Balance
Bioreactor		
	Illumination	Halogen lamps + manual adjustment of intensity
	Feed	Peristaltic pump or stainless steel valves in case of pressure driven transport

	Process water	Process water production station + temperature control + circulation pump
	pH control	Acid-base peristaltic dosing pumps + recipients + balance
	Outlet of reactor content (drain)	Stainless steel valve and piping
	Gas evacuation to the outside in case of overpressure in the bioreactor	Stainless steel mechanical safety Overpressure valve
	Gravimetric determination tank content	Balance
Gas Loop		
	Evacuation of gas produced in bioreactor	Stainless steel and teflon tubing 6 mm and outlet to the outside
	CO_2 and He supply	CO_2 and He bottle, tap, pressure regulator and stainless steel and teflon tubing 6 mm internal diameter
Effluent tank		
	Gas evacuation to the outside in case of overpressure in the influent tank	Stainless steel mechanical safety Overpressure valve
	Slight overpressure with He	Overpressure regulation and stainless steel valve
	Inlet of fresh influent	Stainless steel valve and piping
	Outlet of influent (drain)	Stainless steel valve and piping
	Cooling	external cool process water circuit + Temperature control + circulation pump
Cleaning		
	Outlet (drain) of cleaning buffer	Stainless steel valve and piping
	Gas evacuation to the outside in case of overpressure in the cleaning buffer	Stainless steel mechanical safety overpressure valve
	Water inlet in cleaning agent tank	Stainless steel valves + connections
Sterilization		
	Outlet of steam condensate	Stainless steel valves + connections

6.2.4 System closure

As axeny is aimed for, the content of the reactor and its interfaces will be separated from the environment. Gas tightness will be ensured. The system will be kept under a slight overpressure of 100 mbar. This guarantees that no contamination of the reactor will occur. A mechanical safety overpressure valve will be foreseen.

6.2.5 Sterilisation, maintenance, cleaning and safety issues

Sterilization will occur through a combination of autoclaving and steaming in place. Glass bioreactors needed to obtain 100% illuminated surface are not steam sterilizable. Hence, the selected glass reactor will be sterilized through autoclaving. For the side equipment, sterilization will be through steaming in place.

It is expected that chemical cleaning will only be needed occasionally. The reactor itself can be cleaned rather easily from the top in a manual way, so a complex cleaning in place is not provided. For cleaning in place of pumps, piping and tubing, the chemical solution will be introduced in the influent or effluent vessel manually and then circulated through the lines. Three-way valves will be provided in the influent and effluent line to connect the external pump which is required for circulation of the CIP solution.

6.2.6 Culture strategy

The process requirements to obtain optimal growth of *R. rubrum* have been described in TN 80.16.

As a back-up culture, a volume of 1/10 of the reactor volume will be maintained. This back-up is kept at the same pH, illumination and temperature as in the reactor. Feeding will be done manually.

The freeze-dried *R. rubrum strain* will be revived using R8AH medium (ATCC medium 550), as described in TN37.7. This medium will also be used for routinely subculturing the stock strain.

6.3 System design

6.3.1 Reactor concept

Paragraph 6.3.7 presents a simplified drawing of the photobioreactor.
The photobioreactor is an autoclavable double-walled glass reactor with 100% illuminated volume and with minimal diameter to obtain maximal performances. The inner diameter varies between 16 and 19 cm depending on the supplier. Sensors and ports are located in a stainless steel top plate. The reactor is equipped with Rushton impellers.

6.3.2 Gas injection (CO_2)

Depending on the composition of the influent, carbon dioxide (CO_2) is added to the reactor medium. The addition is performed from a gas cylinder with a certain concentration of CO_2 in a gas mixture with Ar. The addition of CO_2 is controlled at a preset flow and gas flows are registered (see 5.2, option 2).

6.3.3 pH correction of the bioreactor

The desired pH in compartment II is between 6,9 and 7,1. The pH-correction is performed through a pH-measurement in the reactor medium with a combined KCl gel-electrode. An acid or base dosing pump will be activated by an external signal in function of the deviation to the setpoint pH.
For the reactor dimensions of compartment II and with respect to maintenance and simplicity of action, peristaltic pumps are the best choice. Commercial bioreactors have the pH-control together with the dosing pumps integrated into the system.

6.3.4 Illumination and control

The biological process of CII requires illumination and a continuous alteration between light and dark zones. Illumination will occur with halogen lamps. Through manual adjustment of the electrical potential, it will be possible to range the intensities between 0 and 300 W/m². Due to the heat production of the halogen lamps, ventilation around the bioreactor will be needed.

6.3.5 Overpressure control of bioreactor and buffer tanks

The reactor and buffer tanks are kept at a small overpressure of 100 mbar to avoid contamination of the media from the ambient atmosphere. The propellant used will be pressurized helium. The most accurate option for overpressure control is the combination of pressure measurement (transmitter) + electromagnetic valve (proportional) + control loop (see also 5.5).

6.3.6 Feed to the bioreactor

The flow towards the bioreactors is rather small and is estimated to be 12 l/d or 8.3 ml/min at maximum. Accurate flow measurements can be achieved through the combination of a peristaltic pump and weighing of the influent tank with feed back from the weight measurement towards the pump to correct for deviations.

The effluent should be evacuated at a comparable flow rate to the influent flow rate in order to maintain a constant liquid level in the reactor. The most accurate option is to use a combination of a peristaltic pump and weighing of the reactor vessel (see 5.7).

6.3.7 Concept scheme

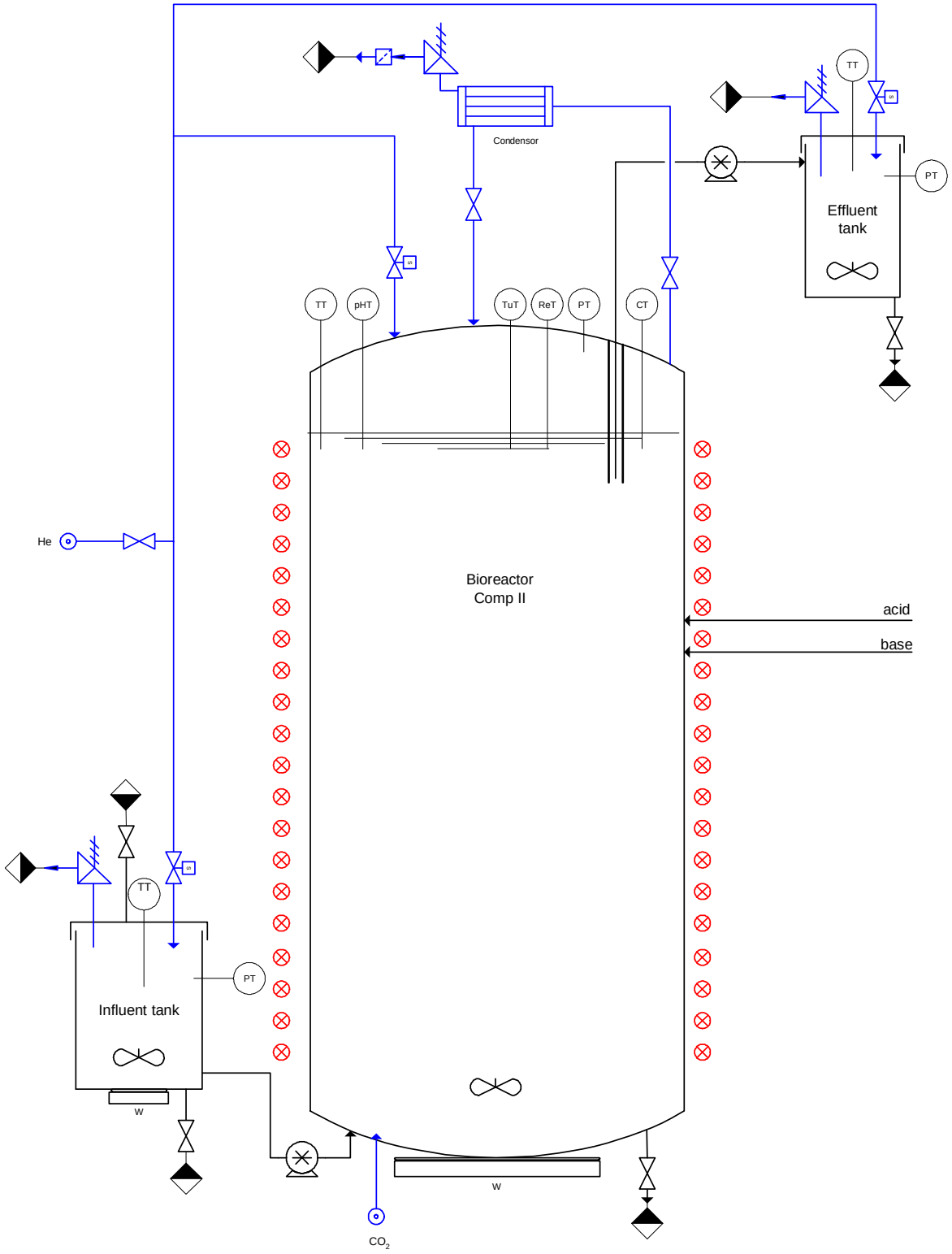

Figure 26: Simplified drawing of reactor CII

7 Compartment III

7.1 Summary of design requirements

The design requirements of this aerobic, nitrifying reactor were described in detail in TN80.16. Table 21 summarizes the technical requirements, related to the reactor itself.

Table 21: Summary of reactor requirements

	Detail	Requirement	Comment
Operation	Recirculation	5:1	recirculation/feed ratio
	Backwash	yes	every 4 to 6 months (TN 47.2)
	Sterility	yes	
	Gas closure	yes	gas release out of reactor due to aeration
	Pressure	100±20 mbar	slight overpressure
	pH-correction	yes	
	Gas flow	constant	to guarantee constant mixing
		Air + x% O_2	to be mixed in correct ratio
			oxygen concentration should be variable
Reactor	Shape	cilindrical	for optimal mixing
	Fixed bed	carrier support	biostyr, glass beads or other
	Stirring	magnetic stirrer 300 rpm	to provide an optimal mixing between inlet and recirculation stream
	Temperature-control	yes	for cooling and heating

7.2 Process characteristics

7.2.1 Sizing

The required active volume of compartment CIII was determined in paragraph 2.2. Its active working volume should be 1 l. Typically, the active volume constitutes 40% of the total reactor volume for a fixed bed system. Hence, the total volume including carriers amounts to 2,5-3 l.

7.2.2 Functioning mode

The reactor will be operated in continuous mode. The reactor will be fed at a fixed flow rate, based on the reactor volume and design dilution rate, and controlled by weighing of the

influent tank. Effluent withdrawal will also be continuous and will be controlled through weighing of the reactor vessel.

7.2.3 Interfaces

The nitrifying reactor will have its own dedicated inlet and outlet storage. Both tanks are provided with a mixing device to guarantee representative sampling. They will be double-walled to allow cooling to 4°C through a central cooling system. The tanks will consist of steam sterilizable stainless steel. The buffering vessels should be sufficient large to cover at least 3 days. This means that the volume of the buffering tanks should be around 40 liters.

As the growth of the nitrifiers will mainly occur as biofilm and growth is slow, only little biomass in the effluent will have to be separated from the liquid. However, the liquid output needs further treatment to guarantee a cell free feeding to the following compartments. The aim of the separation process for this compartment is thus to clarify the liquid. Centrifugation on a liquid of such low cell concentration is too energy consuming. Therefore, membrane technology offers the best filtration. Dead-end filtration is selected because it limits the loss of liquid when operating in closed loop.

The off-gas of the reactor will be monitored, sampled and analysed off-line. For O_2 and CO_2, in-line probes are considered as well (see also 9.3).

Table 22: General overview of the interfaces of CIII

Interface (tag P&ID)	Description	Needed equipment
Influent tank		
	Gas evacuation to the outside in case of overpressure in the influent tank	Stainless steel mechanical safety Overpressure valve
	Slight overpressure with He	Overpressure regulation and stainless steel valve
	Inlet of fresh influent	Stainless steel valve and piping
	Outlet of influent (drain)	Stainless steel valve and piping
	Cooling	External cool process water circuit + temperature control + circulation pump
	Gravimetric determination tank content	Balance
Bioreactor		
	Feed	Peristaltic pump or stainless ssteel valves in case of pressure driven transport
	Process water	Process water production station + temperature control + circulation pump

	pH control	Acid-base peristaltic dosing pumps + recipients + balance
	Recirculation effluent	Peristaltic recirculation pump
	Backwash	Peristaltic backwash pump + stainless steel piping and valves + dead-end filter for biomass removal
	Outlet of reactor content (drain)	Stainless steel valve and piping
	Gas evacuation to the outside in case of overpressure in the bioreactor	Stainless steel mechanical safety overpressure valve
	Gravimetric determination tank content	Balance
Gas Loop		
	Evacuation of gas produced in bioreactor	Stainless steel and teflon tubing 6 mm and outlet to the outside
	Air, O_2, and He supply	Air, O_2, He bottle, tap, pressure regulator and stainless steel and teflon tubing 6 mm internal diameter
Effluent tank		
	Gas evacuation to the outside in case of overpressure in the influent tank	Stainless steel mechanical safety Overpressure valve
	Slight overpressure with He	Overpressure regulation and stainless steel valve
	Inlet of fresh influent	Stainless steel valve and piping
	Outlet of influent (drain)	Stainless steel valve and piping
	Cooling	external cool process water circuit + Temperature control + circulation pump
Cleaning		
	Outlet (drain) of cleaning buffer	Stainless steel valve and piping
	Gas evacuation to the outside in case of overpressure in the cleaning buffer	Stainless steel mechanical safety overpressure valve
	Water inlet in cleaning agent tank	Stainless steel valves + connections
Sterilization		
	Outlet of steam condensate	Stainless steel valves + connections

7.2.4 System closure

As axeny is aimed for, the content of the reactor and its dedicated interfaces should be separated from the environment. Gas tightness is desirable. The system will be kept under a slight overpressure of 100 mbar. This guarantees that in case of possible leakages in the gas loop, no contamination of the reactor will occur. A mechanical safety overpressure valve will be foreseen.
Because of the high risk of contamination for compartment III, there will be no sampling of the carrier material during test runs. This can only be scheduled at the end of a run.

7.2.5 Sterilisation, maintenance, cleaning and safety issues

Sterilization will be achieved through steaming in place of bioreactor, buffering tanks, tubing, … to minimize the risk of microbial contamination. As some carrier materials such as polystyrene beads are not steam sterilisable, chemical sterilization will have to be applied under those conditions.
As chemical cleaning will most probably only be needed occasionally, a complex cleaning in place is not needed. The reactor and buffering tanks can easily be cleaned manually from the top. The packed bed is removable from the top and can be replaced or cleaned separately. Inlet and outlet tubings will be easy to disconnect from the bioreactor in view of optimal cleaning. Cleaning in place is recommended for pumps and tubing. This will be achieved through circulation of CIP solution from the influent or effluent buffer tank. To this end, manual three-way valves will be provided in the influent and effluent lines. One and the same external pump will be used for CIP of all compartments.

7.2.6 Culture strategy

Because of the slow growth of both nitrifying organisms, their cultivation to large volumes will be initiated one year before start-up of compartment I is planned. Inoculation of the carrier material will be done through continuous batchwise recirculation of the cell suspension over the carriers, according to the start-up protocols described in TN37.510.
Once sufficient biofilm is obtained, there will be a switch from batch to continuous operation.

A back-up culture will be transferred to fresh standard medium at regular time intervals. Its target volume is 10% of the bioreactor liquid volume.

7.3 System design

7.3.1 Reactor concept

The reactor will be an in-line steam sterilisable stainless steel packed-bed system. A sight-glass is provided for visual observation of the carrier material.
The active liquid volume was calculated to be 1 l based on the hydraulic retention time. The volume of the reactor carrier material included is ~3 l. On top and below the carrier zone, additional liquid space is foreseen for entering the probes.
The influent is fed at the bottem of the reactor and flows upward through the packed bed. The effluent on top is pumped out of the reactor. Another pump recirculates the effluent back to the bottom space of the reactor at a recirculation:feeding ratio of 5:1.

7.3.2 Fixed bed

Glass or polystyrene beads will be used as carrier material. After a certain period of operation, the biofilm will have grown on the carriers and clogging of the fixed bed could occur. This

process will be monitored by means of two manometers, respectively in the head and bottom space of the reactor. A back-wash will be required at that time.

7.3.3 Aeration device

Compartment CIII is an aerobic bioreactor. Therefeore, oxygen limitation must be avoided. As For aeration purposes, air will be supplied, to which oxygen can be dosed, when required. During start-up, at low biomass concentration, the oxygen demand will be rather low. As the biofilm becomes more dense, oxygen demand will increase and additional supply with pure oxygen may be needed. As a plug flow may occur through the packed bed, oxygen requirements will vary along the bed height. To avoid oxygen limitation, oxygen supply should be controlled through oxygen concentration measurements in the effluent.

In any case, the gas flow rate to the bioreactor must be kept constant to avoid changes in flow patterns through the packed bed. The control of the constant flow rate through the reactor can be done by means of thermal mass flow measurements. Two different solutions are given:

1. *The control of the total gas flow is independent of the control system of the bioreactor:* Figure 27 illustrates this option.

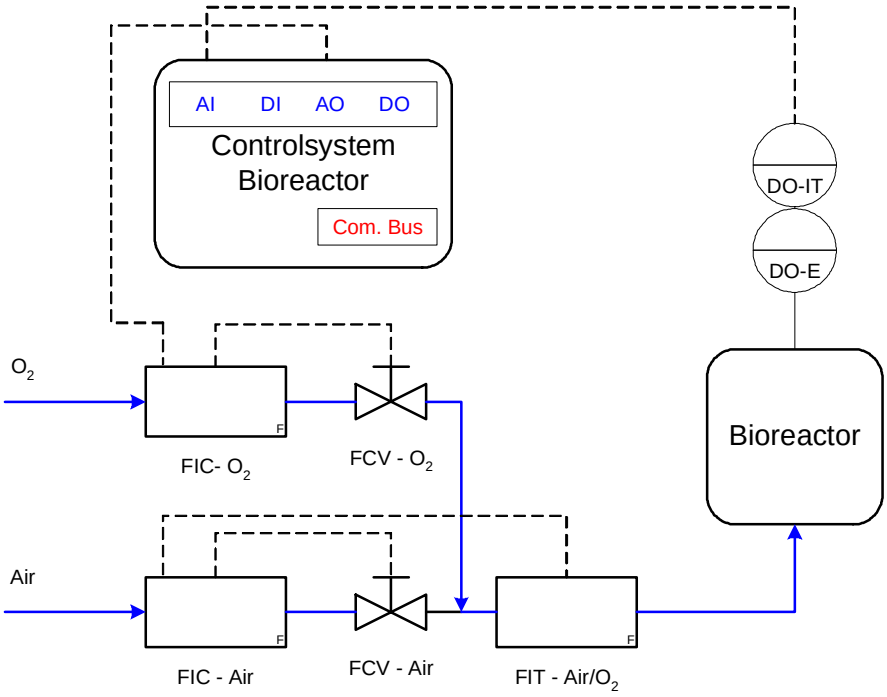

Figure 27: Independent control of the total gas flow

The thermal mass flow controller will open a regulation valve at a certain position, dependent on the oxygen requirement in the bioreactor. As a result, the total flow to the bioreactor will fluctuate. These variations should be excluded by means of a flow measurement on the mixed gas stream. Deviations from the required total flow rate are compensated through a mass flow controller and regulation valve on the air flow.
An important disadvantage of this system lies in the fact that a mass flow measurement is sensitive to changes in thermal conductivity and the heat capacity of the gas mixture. A change in the composition O_2/air has an impact on these parameters and, as a result, will lead to deviation from the correct flow measurement. The error on the measurement will be rather small as air and oxygen have similar characteristics. The maximal fault on the measurement will be 1 to 2%.

2. *The control of the total flow is done by the control system of the bioreactor*: Figure 28 illustrates this option.

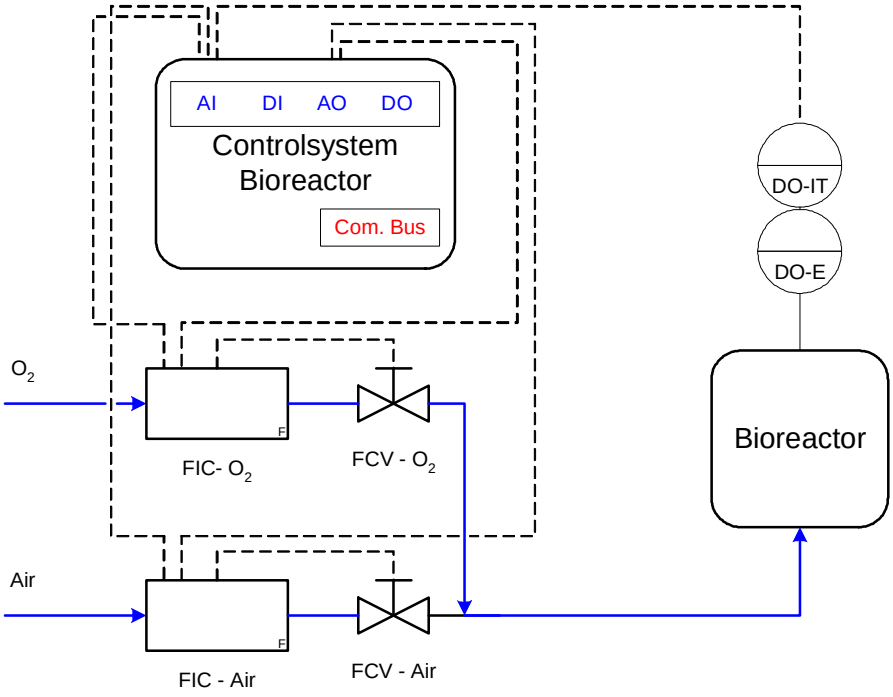

Figure 28: Control of the total gas flow through the control system

The difference compared to option 1 is that the final control is not done through a separate flow measurement, but through the control system of the bioreactor. The sum of the air flow rate and oxygen gas flow rate should equal the required total flow rate through the bioreactor permanently. In case of a deviation, the thermal mass flow measurement will adjust the regulation valve on the air supply.

Remarks and conclusions:
In case the control system of the bioreactor can be easily configurated, option 2 offers the most interesting solution since no mass flow measurement on the total gas flow is needed and no error as in option 1 occurs.

7.3.4 Recirculation and Backwash

In order to avoid excessive biofilm growth and clogging at the entrance of the fixed bed, a recirculation over the reactor is maintained. The ratio between the recirculation rate and the inlet flow is fixed (~ 5:1). The higher flow rate results in a shear which limits biofilm thickness and may slough off parts of the biofilm. The effluent is sent back to the inlet of the reactor and is injected over the complete diameter of the reactor. Recirculation is achieved by means of a peristaltic pump.

After a certain period of time, the fixed bed will clog due to an excess of biomass. This will be detected by means of a pressure drop measurement over the fixed bed. At that moment, a back-wash cycle (see alsoTN 47.2) must be initiated. This can be done in two different ways:

1. The pumping direction of the recirculation pump can be changed for a certain period of time. This is performed manually. By reversing the flow direction the biomass is released from the carrier material. During the backwash, the medium is sent through a filtration to avoid that the released biomass enters the bioreactor again. The collected biomass will then be removed from the reactor (see Figure 29).

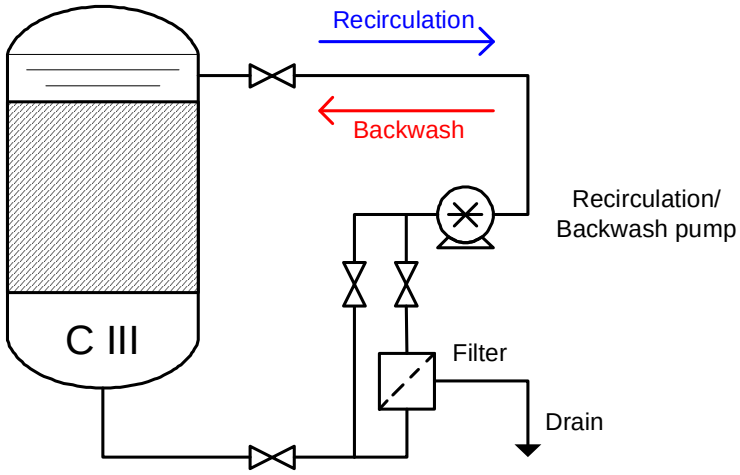

Figure 29: Backwash or recirculation

2. This option offers a more complicated back-wash cycle. Effluent is pumped back to the bioreactor by means of a dedicated pump or by changing the pumping direction of the effluent evacuation pump. The recycle on the bioreactor is switched off and the washing

water, containing biomass, is rinsed out towards a dedicated storage tank (see Figure 30). This implies a loss of effluent which is not desirable for closed loop operation.

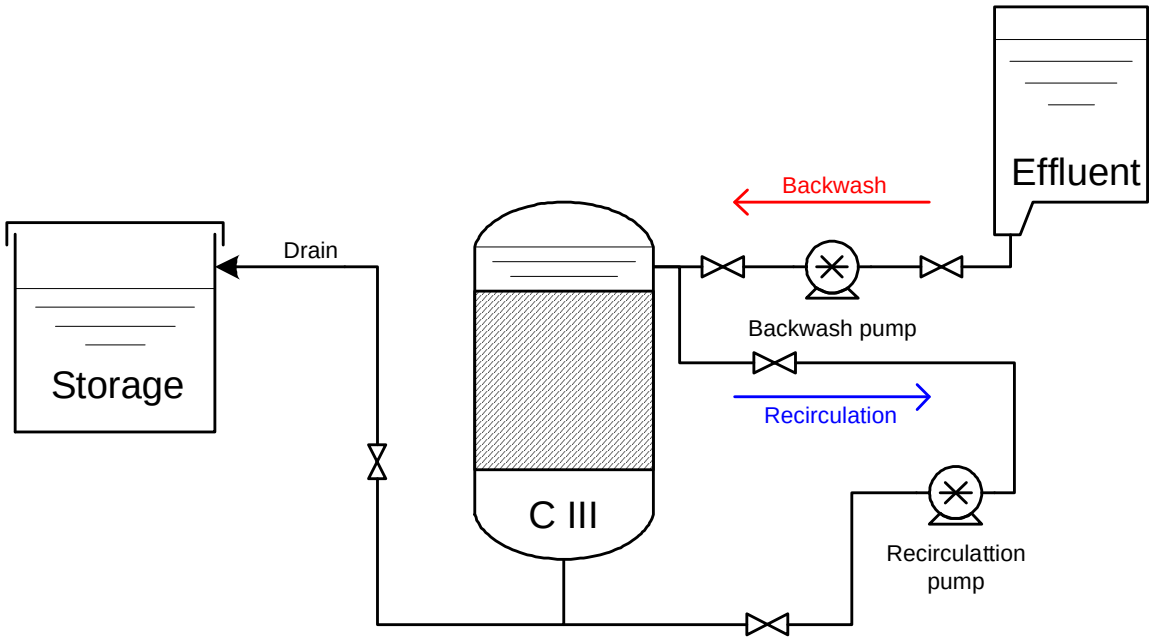

Figure 30: Backwash and recirculation

Option 1 is the best choice due to simplicity and reduced costs. Furthermore, advantage can be taken from the filtration to collect biomass-free samples.

7.3.5 Temperature control bioreactor (and buffer tanks)

The temperature in the reactor and the buffer vessels will be measured with Pt100 sensors. The different options for temperature control have been described in paragraph 5.1.

The influents and effluents of the bioreactor must be cooled to conserve the media. The desired temperature is 4°C and cooling down to this temperature will be achieved through a double-walled tank design. The cooling liquid will be generated from a central cold process water system.

For compartment III the setpoint is 30°C. Contrary to the photobioreactors which need cooling, this fixed bed reactor must be heated. A continuous precise regulation of the temperature requires individual temperature control. This will be achieved through a temperature measurement in the medium of the bioreactor with feedback towards an individual system of temperature control. Because the heating requirements are limited, the double jacket will only cover part of the reactor vessel.

7.3.6 pH correction of the bioreactor

The desired pH in compartment III is 8,15±0,15. The pH-correction is controlled by the pH measurements in the reactor. Two pH probes are foreseen, one at the top and one at the bottom of the reactor. A pH difference can be expected as a result of:

- ✓ acidification due to the nitrification reaction,
- ✓ pH increase due to the stripping of CO_2.

The pH control will be based on an average value of both measurements.
A combined gel-electrode (i.e. a glass electrode with built in reference electrode) is used.

A peristaltic base (and acid) dosing pump will be activated by an external signal in function of the deviation to the setpoint pH. Commercial bioreactors have the pH-control together with the dosing pumps integrated into the system. Addition of acid or base will take place in the recirculation line.

7.3.7 Overpressure control of bioreactor and buffer tanks

The control and pressure system for the buffering tanks will be identical to the one described for CII in paragraph 6.3.5. The option with the pressure measurement, electromagnetic proportional valve and the control loop offer the most accurate solution for the buffering tanks, but will be more expensive. The reactor itself only needs an overpressure release valve set at a certain overpressure value.

As the reactor is aerated, an overpressure can be maintained by means of an overpressure release valve. No additional injection of an inert gas is needed.

7.3.8 Feed to the bioreactor

The flow to the bioreactor is rather small and is estimated around 10-12 l/d or 8,3 ml/min. A gravimetric follow up of influent and reactor vessel will be used to control influent and effluent flow rate (see also 6.3.6).

7.3.9 Concept scheme

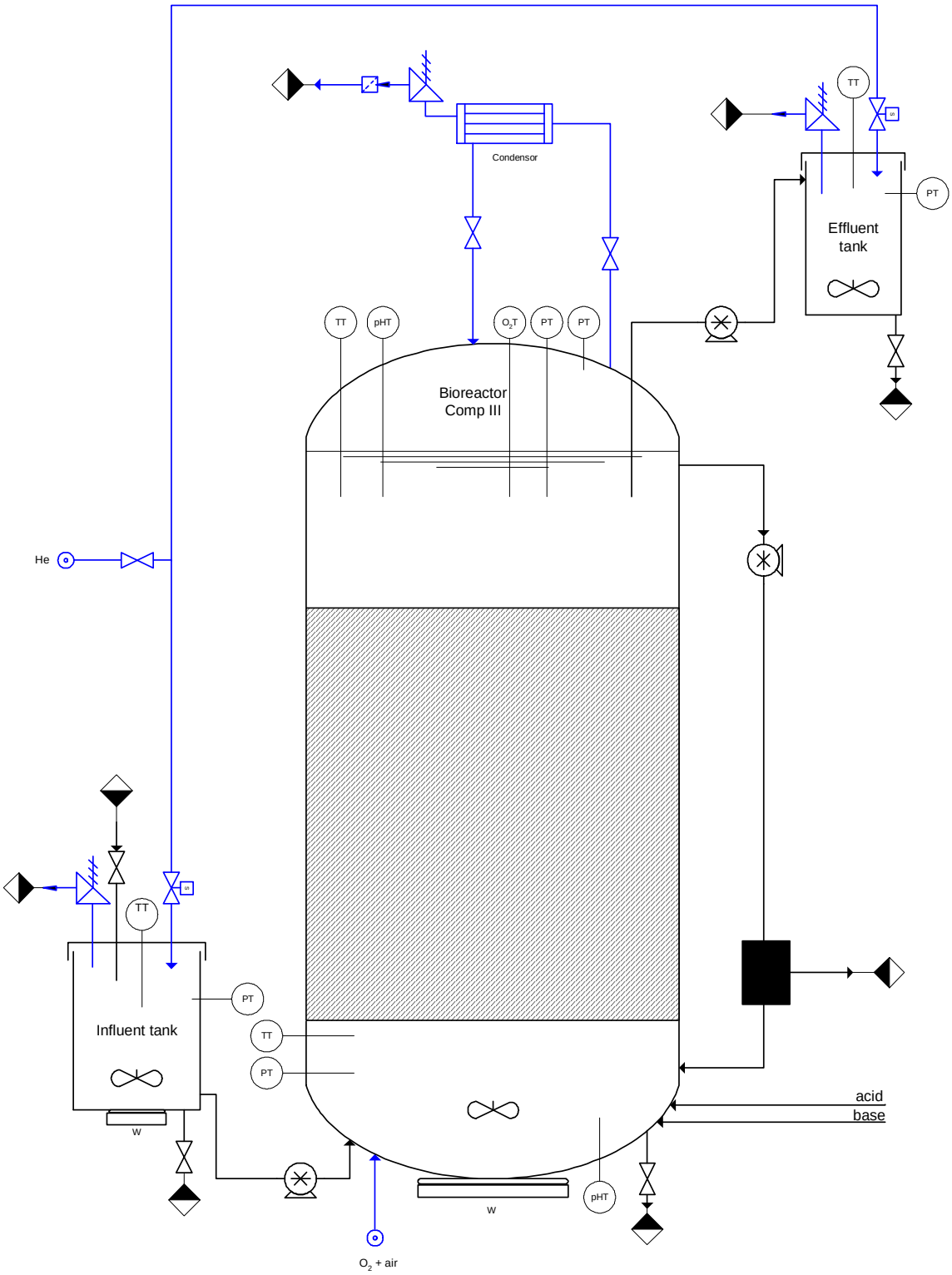

Figure 31: Simplified drawing of reactor CIII

8 Compartment IVa

8.1 Summary of design requirements

The design requirements for this mesophilic photosynthetic reactor were described in detail in TN80.16. Table 23 summarizes the technical requirements, related to the reactor itself.

Table 23: Summary of reactor requirements

	Detail	Requirement	Comment
Operation	Sterility	yes	
	Gas closure	yes	controlled gas release out of reactor due to aeration
	Pressure	100±20 mbar	slight overpressure
	pH-correction	yes	
	Gas flow	air + x% CO_2	in case of closed loop operation. For tests with Zarrouk medium sufficient bicarbonates are available in the medium.
Reactor	Shape	cylindrical	for optimal mixing and illumination
	Diameter	small	to avoid dark zones in centre dark zones at headspace should be minimal as well
	Illumination	0 - 300 W/m²	excess light becomes inhibitory
		variable	avoid dark surfaces and zones
	Stirring	good radial homogeneity	mechanical stirrer: easy to control
		max. 300 rpm	mixing with gas flow could be possible as well
	Cooling	yes	due to the irradiation of the reactor cooling is required

8.2 Process characteristics

8.2.1 Sizing

The required active volume of compartment CIVa was determined in 2.2. In case of intensive sampling a volume around 16 l was suggested.

8.2.2 Functioning mode

The reactor will be operated in continuous mode. The feeding of the reactor will be done at a fixed flow rate, based on the reactor volume and design dilution rate. The influent and effluent flow rates will be controlled by weighing of the influent tank and reactor vessel respectively.

8.2.3 Interfaces

The photobioreactor CIVa will have its own dedicated inlet and outler storage. Both tanks have a mixing device. Especially the effluent vessel, containing a suspension of algae, should be homogeneous to guarantee representative sampling. Furthermore, the buffering vessels will be cooled to 4°C through a double jacket and a centralised cooling system.

A solid-liquid separation or biomass harvesting system will be needed to separate the *Arthrospira platensis* from the effluent. Within the contract 'MELiSSA Space Adaptation', an extensive literature review was performed to identify suitable solid-liquid separation systems for the algae suspension of compartment IVa (TN72.7.2) . These were centrifugation, membrane filtration and acoustic separation. Finally, a combination of acoustic separation and membrane filtration was selected to be optimal for MELiSSA. For BELISSIMA, it is less important to maintain cell integrity and to preserve the nutritional quality of the cells. Therefore, since acoustic separation can only achieve a limited concentrating effect, preference is given to membrane filtration. This would ideally require ceramic ultrafiltration membranes operated in crossflow. Cross-flow membrane filtration, however, has the important disadvantage that liquid is wasted through the concentrate.

The off-gas of the reactor will be monitored by sampling the gas, followed by analysis or through direct on-line measurements. On-line analysis of the gas phase s highly recommended.

Table 24: General overview of the interfaces of CIVa

Interface (tag P&ID)	Description	Needed equipment
Influent tank		
	Gas evacuation to the outside in case of overpressure in the influent tank	Stainless steel mechanical safety Overpressure valve
	Slight overpressure with He	Overpressure regulation and stainless steel valve
	Inlet of fresh influent	Stainless steel valve and piping
	Outlet of influent (drain)	Stainless steel valve and piping
	Cooling	external cool process water circuit + Temperature control + circulation pump
	Gravimetric determination tank content	Balance
Bioreactor		

	Illumination	Halogen lamps + manual adjustment of intensity
	Feed	Peristaltic pump or stainless ssteel valves in case of pressure driven transport
	Process water	Process water production station + temperature control + circulation pump
	pH control	Acid-base peristaltic dosing pumps + recipients + balance
	Outlet of reactor content (drain)	Stainless steel valve and piping
	Gas evacuation to the outside in case of overpressure in the bioreactor	Stainless steel mechanical safety Overpressure valve
	Gravimetric determination tank content	Balance
Gas Loop		
	Evacuation of gas produced in bioreactor	Stainless steel and teflon tubing 6 mm and outlet to the outside
	Air, CO_2 and He supply	Air, CO_2 and He bottle, tap, pressure regulator and stainless steel and teflon tubing 6 mm internal diameter
Effluent tank		
	Gas evacuation to the outside in case of overpressure in the influent tank	Stainless steel mechanical safety Overpressure valve
	Slight overpressure with He	Overpressure regulation and stainless steel valve
	Inlet of fresh influent	Stainless steel valve and piping
	Outlet of influent (drain)	Stainless steel valve and piping
	Cooling	external cool process water circuit + Temperature control + circulation pump
Cleaning		
	Outlet (drain) of cleaning buffer	Stainless steel valve and piping
	Gas evacuation to the outside in case of overpressure in the cleaning buffer	Stainless steel mechanical safety overpressure valve
	Water inlet in cleaning agent tank	Stainless steel valves + connections
Sterilization		
	Outlet of steam condensate	Stainless steel valves + connections

8.2.4 System closure

As axeny is aimed for, the content of the reactor and its dedicated interfaces should be separated from the environment. The system will be gas tight and will be kept under a slight overpressure of 100 mbar. This guarantees that in case of possible leakages in the gas loop, no

contamination of the reactor will occur. A mechanical safety overpressure valve will be foreseen.

8.2.5 Sterilisation, maintenance, cleaning and safety issues

Sterilization will occur through a combination of autoclaving and steaming in place. Completely illuminated glass bioreactors are autoclavable but not steam sterilizable (see also 8.3.1). Hence, the bioreactor will have to be dismounted, then sterilized in an autoclave and finally reconnected. All side equipment will be SIP sterilizable.

All equipment will be easy to clean and sterilize. A complex cleaning in place is not required. The reactor and buffer vessels can be easily cleaned from the top. For optimal maintenance of the reactor the inlet and outlet tubings will be easy to disconnect by manual valves. To chemically clean pumps and tubing, a chemical solution will be introduced manually in the influent or effluent vessel and circulated with an external pump through the lines.

All equipment and valves, needed for the cleaning and steaming in place will be indicated on the drawings and P&ID's.

8.2.6 Culture strategy

The process requirements to obtain optimal growth of *Athrospira platensis* have been described in TN80.16.

As a back-up culture, a volume of 1/10 of the reactor volume will be maintained at comparable condition as in the reactor itself. As the algae concentration will double more or less daily, the solution will have to be refreshed frequently.

8.3 System design

8.3.1 Reactor concept

The discussion of the reactor concept is to some extent similar to the one for compartment II (see 2.1.3 and 6.3.1). The main difference is that compartment IVa will have a larger working volume (16 l compared to 10 l) and this has implications with respect to the availability of autoclavable reactors with optimal illuminated volumes. The following options are commercially available:

- ✓ Double jacketed glass bioreactor: up to 10 liters working volume
- ✓ Single jacketed glass bioreactor: up to 15 liters working volume
- ✓ Polymer bioreactors

1. *Autoclavable bioreactors:* glass reactors are available which are autoclavable. Working volumes are limited to 10 l for double jacketed designs. Some suppliers exceptionally provide autoclavable glass bioreactors with a working volume up to 15 l, but these are

single jacketed. Clearly, such volumes and weights are very difficult to handle, manipulate for autoclaving etc.

2. *Polymer bioreactors:* if no autoclavable glass bioreactors are available at the desired working volume, membrane film bioreactors may be a suitable alternative. The reactor wall consists of a polyamide film which is clamped between stainless steel flanges which contain ports and connections for sensors, mixing devices etc. The advantage of these reactors is that they are in situ sterilisable and can be obtained at any working volume. A clear disadvantage in the frame of micrcompound investigations, is that the use of plastic materials should be minimized to avoid leaching of contaminants. Additionally, the illumination of the foils with halogen lamps with high heat dissipation can raise issues in terms of safety and operational continuity. The foil should have a certain heat resistance of its own since cooling through a double jacket is not an option.

Suppliers:

✓	Autoclavable bioreactors (max. 10 l):	Bioengineering, Sartorius, New Brunswick, Applikon;
✓	Autoclavable bioreactors (> 10 l):	Applikon;
✓	Polymer bioreactors:	Bioengineering

Preference is given to glass reactors. The bioreactor will be a stirred tank concept.

As opposed to CII, there will be constant dosing of CO_2 (except when sufficient bicarbonate is present in synthetic media) and that the dissolved oxygen concentrations will be monitored in the medium.

8.3.2 Aeration and CO_2 injection

Aeration is meant to provide CO_2. If the supply is insufficient, additional CO_2 will have to be supplemented. The addition is performed from a gas cylinder. Since the reactor is not conceived as an airlift but as a stirred tank, there is no need for a constant flow. As for compartment II, the addition of CO_2 will be controlled at a preset flow with registration of gas flows (see also 5.2, option 2).

8.3.3 Temperature control bioreactor (and buffer tanks)

The temperature in the reactor and the buffer vessels will be measured with Pt100 sensors. The different options for temperature control have been described in paragraph 5.1.

The temperature of the bioreactor will be regulated through an individual temperature control system and double jacket at 36±0.5°C.

The influents and effluents of the bioreactor must be cooled to conserve the media. The desired temperature is 4°C and cooling down to this temperature will be achieved through a double-walled tank design. The cooling liquid will be generated from a central cold process water system.

8.3.4 pH correction of the bioreactor

The desired pH in compartment IVa is 9.5. The pH-correction is performed through a pH-measurement in the reactor medium with a combined KCl gel-electrode. An acid or base dosing pump will be activated by an external signal in function of the deviation to the setpoint pH. Dosing pumps will be peristaltic ones. Commercial bioreactors have the pH-control together with the dosing pumps integrated into the system.

8.3.5 Illumination and control

The biological process of CIVa requires illumination with halogen lamps. Through manual adjustment of the electrical potential, it will be possible to range the intensities between 0 and 300 W/m². Due to the heat production of the halogen lamps, ventilation around the bioreactor will be needed.

8.3.6 Overpressure control of bioreactor and buffer tanks

The reactor and buffer tanks are kept at a small overpressure of 100 mbar to avoid contamination of the media from the ambient atmosphere. This will be achieved through the aeration and CO_2 injection. The most accurate option for overpressure control is the combination of pressure measurement (transmitter) + electromagnetic valve (proportional) + control loop (see also 5.5).

8.3.7 Feed to the bioreactor

The flow to the bioreactor is estimated around 10-12 l/d or 8,3 ml/min. A gravimetric follow up of influent and reactor vessel will be used to control influent and effluent flow rate.

8.3.8 Concept scheme

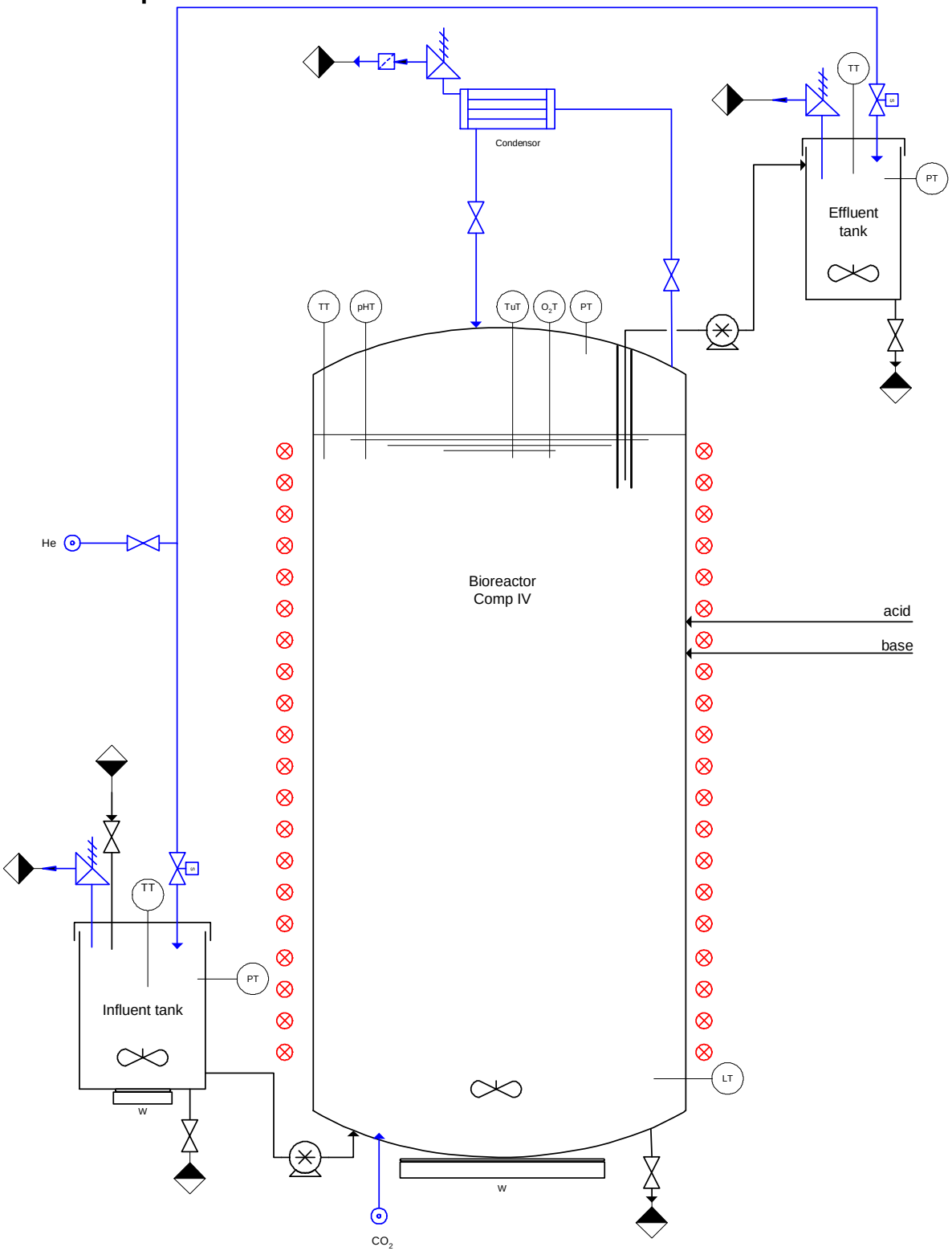

Figure 32: Simplified drawing of reactor CIVa

9 Overall interfaces

9.1 Steam production

All equipment of the BELISSIMA loop downstream the compartment I bioreactor will be in situ steam sterilizable, except for the glass bioreactors required for compartments II and IVa. Steam production will be achieved through a portable steam production unit, which can be connected to 3 way-valves inserted in different subcompartments of the loop.

9.2 Autoclave

The bioreactors of compartment II and IVa will have to be sterilized in a separate autoclave. The choice of the autoclave will depend on the final width and height of the bioreactors, including sensors and ports. Several options are commercially available.

9.3 Gas analyses

For CO_2 and O_2 measurement, 2 options are available. Either the bioreactors will be equipped with in-line probes or a separate gas processing and analysis unit will be available, which allows for automated intermittent sampling and analysis of each compartment. For other parameters, the analyses will be off-line.
The gas phase of compartment I will be sampled and analysed off-line.

9.4 Cooling equipment

It has been mentioned several times in this TN that the cooling of the influent and effluent tanks of compartments II to IVa will be achieved through a common central cooling device. This will be designed to have sufficient cooling capacity. To avoid capacity losses, all piping will be thoroughly insulated and the distance from the central cooling system to the different tanks will be minimized. Probably, it will not be possible to position compartments II to IVa and their influent and effluent tanks at very short distances. If this results in too long pipings, a second cooling unit may be implemented at a later stage of the BELISSIMA loop assembly.

9.5 CIP

Because it is expected that a CIP will only be required occasionally, there is no need for a complex CIP system. Reactor and buffer vessels will be cleaned manually from the top. Inaccessible lines will be rinsed with a chemical solution, which will be added manually to the influent or effluent tank. A stand-alone CIP unit will be available which can be stepwise connected to each subcompartment to circulate the chemical solution, if needed.

10 Addendum

10.1 Simulations by UBP: first simulation BELISSIMA simplified loop

Based on C1 + C2 + C3 + C4a – MELiSSA 0.10 model
Loop closed for liquid with control on flow rate and composition on C1
Loop open for gas without external supply

Input setting

C1 inputs :

Urine : water = 9 L/day = 9 kg/day
urea = 0.17 kg/day

Faeces : water = 0.36 kg/day
solid = 0.18 kg/day

Composition $CH_{1.526}O_{0.327}N_{0.2496}S_{0.0002}P_{0.0003}$
10 % proteins, 20 % fats, 10 % carbohydrate, 60 % fibers

Organic Matter (plants) : water ≈ 20 kg/day
solid ≈ 2 kg/day

Composition $CH_{1.526}O_{0.327}N_{0.2496}S_{0.0002}P_{0.0003}$
15 % proteins, 7 % fats, 1 % carbohydrate, 77 % fibers

C3 inputs :

Gas (air) input (for aerobic compartment)
0.32 kg O_2/day
1.06 kg N_2/day
that is to say around 1 m^3 air/day

Compartments operation setting

C1 :
- no acetogenesis, no methanogenesis
- 80 % degradation of proteins
- 80 % degradation of fats
- 100 % degradation of carbohydrate
- 20 % degradation of fibre into carbohydrate
- 50 % recovery of minerals (N, S, P)from fibre
- 100 % degradation of urea

C2 : 100 % assimilation of VFA
C3 : 100 % oxydation of NH_3 to HNO_3
C4 : 100 % assimilation of HNO_3

Results

Operating C I v. 0.0.5 ----------

Note : S and P elements > discrepency Orignal OM / standardised CHONSP
Note : Problem in Fiber degradation for high content in waste recycling
C 1 : Initial OM composition ---------
Proteins : 0.14605
Lipids : 0.080263
Carbohydrates : 0.017105
C 1 : Computed OM composition Matching Standart CHONSP---------
While 1 correction 1
Proteins : 0.14605
Lipids : 0.080263
Carbohydrates : 0.017105
Remaining : 0.75658 C0.070694 H0.090223 O0.043371 N0.0047702 S0.00036348 P0.00045476
.....Composition correction factor : 1
C 1 : Computed OM composition With Fiber decomposition---------
While 2 correction 1
Proteins : 0.14802
Lipids : 0.081346
Carbohydrates : 0.17069
Non degradable: 0.59994
.....Total Hydrolysis correction factor : 1
While 3 : Final Residual OM correction 1
C 1 : Mass bal.=2.3295e-005// Mass Rel.=2.2838e-007// MS Rel.=-0.0028741
-- Dynamic estimations assumming mass flows in kg/day :
Liq flow rate:99.3183 L/day Gas Flow rate:107.8014 NL/day
Net Degradation:0.31286
Theroretical OM load:0.0021kg/L/day Sim. OM conc:0.022956kg/L
Theroretical HRT:10 days
Required flow rate:108.5714 L/d **Estimated Vol:1085.7143 L**
Fin operation CI ----------

Operating C II v. 0.0.3 ----------

Bilan Masse C II = -5.6843e-014 Relative masse balance=-5.6599e-016
-- Dynamic estimations assumming mass flows in kg/day :
Liq flow rate:98.965 L/day Gas Flow rate:496.916 NL/day
Theroretical productivity:0.0021kg biomass/L/day Sim. biomass produced:0.62816kg/day
Estimated Vol:299.1219 L Theroretical HRT:3.0225 days
Stop Operating C II v. 0.0.1 ----------

Operating C III v. 0.0.2 ----------

Bilan Masse C III = -1.4211e-014 Relative masse balance=-1.4058e-016
-- Dynamic estimations assumming mass flows in kg/day :
Liq flow rate:99.2756 L/day Gas Flow rate:12401.6977 NL/day
Net efficiency nh3->no3:0.98387
Theroretical NH3 load:0.0016kg/L/day Sim. nh3 conc:0.00077163kg/L/day
Sim theo HRT:0.48227 days
Estimated Vol:47.8775 L
Stop Operating C III v. 0.0.1 ----------

Operating C IVa v. 0.0.2 ----------

Reaction limited by other compound : 9... New key calculated : 0.16734
Bilan Masse C IVa = 5.6843e-014 Relative masse balance=5.6237e-016
-- Dynamic estimations assumming mass flows in kg/day :
Liq flow rate:98.9367 L/day Gas Flow rate:12741.2265 NL/day
Theroretical productivity:0.00144kg biomass/L/day Sim. biomass produced:0.11808kg/day
Estimated Vol:81.9988 L Theroretical HRT:0.8288 days
Stop Operating C IVa v. 0.0.1 ----------

CARE :
Not enough CO_2 for C4a, so, only 17% of HNO_3 is assimilated (the objective is to reach 100 %)
Verify HRT in reactors after the C1 and verify the compatibility with the specifications fixed (productivity)

10.2 Nomenclature and symbols

Tag: L S – G – 0 2

- L: F: Flow; L: Level; P: Pressure; pH: pH; S: Turbidity; T: Temperature
- S: I: Indicator; S: Sensor; T: Transmitter
- G: V: Feeding system; R: Bioreactor; F: Filtration Unit; G: Gas Loop; C: Cleaning system; S: Sterilization system

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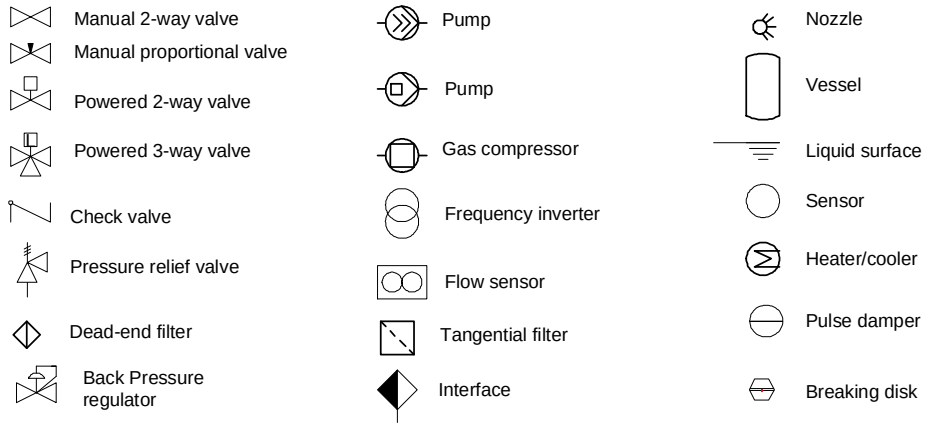
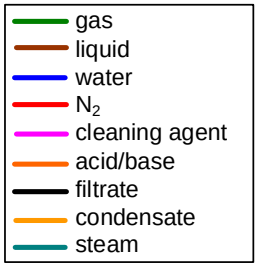
Ec: Electroconductivity
pH: pH
A: Analyser

R: Reactor/tank
V: Valve
BL: Blender
Fi: Filter
HX: Heat exchanger
No: Nozzle
PMP: Pump
St: Steam generator
PR: Pressure regulator

Symbols:





Comments on TN 80.21-23
Compartment design - Associated instrumentation and control hardware-Interfaces between compartments

General comments

This TN is a comprehensive document on compartment design. Some issues are not addressed in details (e.g. instrumentation selection…), most probably due to the supplying strategy followed (commercial standard hardware, global delivery of vessels/instrumentation/control hardware via one supplier).
However due to the financial issue faced today, this level of detail will be probably reached only at the level of detailed design (i.e. in TN 80.24 and design review datapackage).
As a consequence, we will approve this TN, provided that our detailed comments are addressed, without asking a deeper level of information.

Detailed comments

page	paragraph	comment
21	2.2.4	"it is not expected that the VFA production would be lower" This statement is not fully consistent with "the uncertainty on the degradation efficiency is high" as mentioned on page 17 , paragraph 2.2.3. Clarified in TN.OK
23	2.2.5.6	This issue will have to be studied carefully and discussed before any decision on any countermeasure. Noted.OK
26	2.2.6	We do not follow your reasoning: "if 0.5 l sample is removed after CI, this should be compensated by increasing the dry mass input to CI". Please clarify If the liquid flow is increased from 1.6 to 2.1 l/d, why do you mention "almost doubled to 2.1 l/d" ?(see page 37 as well) Clarified in TN.OK
27	3.2	UAB is presently working on the same issue. Information should be shared. Discussion is ongoing.
36	4.1	On-line analysis of the gas phase is highly recommended. With off-line analysis, accuracy of mass balance calculation will be jeopardize (major issue for C). High costs of implementation. Average values on one day basis provide already relevant data for mass-balances. We do not agree, the cost of experiments potentially "lost" is by far higher, we do not have the same feed than for CI in the MPP and therefore need to follow accurately the process performances
36	4.2.1	some figures are not fully consistent along the TN (e.g. dilution ratio 5-fold on page 21) Clarified throughout TN.OK
47	5.6	Sartorius sent some information to UAB with regards to pumps working at low flows. Feed-back has been asked to UBP. Information should be shared.

		Peristaltic lab-scale pump should be ok for range of Belissima flow rates. Info will be shared with UAB and UBP.OK
51	5.9	We do not foresee the use of centrifugation for CIII effluent. In the MELiSSA Pilot Plant, advantage will be taken from the recirculation loop to implement filtration . Noted and clarified in TN.OK
60	5.9	We do not follow your reasoning; you are currently working on membrane filtration for Rubrum Harvesting but here you conclude that cross-flow filtration should not be used? Aiming at a one step process and minimal volume loss, cross-flow filtration is less interesting. The technology although is suitable. Specified in TN.OK
63	Remarks and conclusions	We do not share your point of view with regards to cost efficiency of the control systems sold by bioreactors manufacturers. This topic will further be addressed in TN80.24.Please reword the paragraph to reflect that the systems “might “be more compact……and that the various supplying alternatives will be further addressed in TN 80.24.
76	Figure 28	Drain is missing on your scheme, isn’t it? Which option do you intend to use? Drain is indeed missing on the indicated filtration step (=concentrate). Will be indicated. Option 1 appears best choice, due to simplicity and reduced costs. Advantage could be taken from the filtration to collect biomass-free samples,
81	8.2.3	On-line analysis of the gas phase is highly recommended, for mass-balance issues. High costs of implementation. Average values one day basis provide already relevant data for mass-balances. Later implementation in case of clear necessity will be possible.We would like to have some figures about the implementation costs before taking a final decision