MELiSSA

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HACCP ANALYSIS AND PREPARATION OF BOTH METHANOGENESIS UNITS PRIOR TO ASSAMBLY

(PROJECT: A TOTAL CONVERTING AN BIOSAFE LIQUEFACTION COMPARTMENT FOR MELISSA)

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1 INTRODUCTION AND OBJECTIVES

A first objective of the fourth technical note of the second phase of this project was on the HACCP (Hazard Analysis Critical Control Point) analysis of the methanogenesis units. HACCP is a preventive method which identifies and evaluates the hazards associated to the various stages of a process, and defines the means necessary for their control. HACCP analysis was performed following the hazard analysis protocol, based on the ECSS-Q-40-02A report (2003) on Space Product Assurance - Hazard analysis. In a second phase, critical control points (CCPs) and their critical limits were determined and a monitoring system was established.

Secondly, lab-scale methanogenesis units for assembly were constructed. In this phase of the project the high-load methanogenic reactor was run at a solid retention time of 40 d, as recommended by the outcome of the results in TN 1.7. The performance of the reactor under these conditions was monitored.

Finally, a sixth closed loop experiment was performed with the methanogenic biofilm reactor treating the material from the *Fibrobacter* unit followed by the subcritical hydrothermolysis treatment.

The tasks described for this TN are given below:

INPUTS

- Operational high-load and low-load methanogenesis unit
- Required analysis equipment for COD-analysis, DM-analysis, ammonia analysis
- Hazard analysis of both digesters

Tasks included

- Definition of CCPs and critical limits
- Establishment of monitoring procedures (pathogens; molecular probes, conventional plating and toxic compounds; chemical analysis) for HACCP analysis
- Construction of lab-scale methanogenesis for assembly
- Substrate exchange with Partner 2 and Partner 4 (solid digester residue)
- Batch experiments with supernatant from Partner 2 and hydrolysate from Partner 4

2 HACCP OF METHANOGENESIS UNTIS

The objective of the HACCP method is to guarantee the safety and to set up the quality control of a product. It led to identify seven principles of action:
- To conduct hazard analysis and identify preventive measures,
- To identify critical control points (CCPs) in the process,
- To establish critical limits,
- To monitor each CCP,
- To establish corrective actions,
- To establish verification procedures,
- To establish record-keeping and documentation procedures.

HACCP analysis is generally performed in 3 phases:
Phase 1: Hazard analysis
Phase 2: CCP determination
Phase 3: Establishment of a monitoring system.

Phase 1 : Hazard analysis

Hazard analysis was based on the ECSS-Q-40-02A report (2003) on Space Product Assurance – Hazard analysis.

HAZARD ANALYSIS CONCEPT

The general concept of hazard analysis is depicted in Figures 1-4.
Hazards which are present through hazard manifestations in the system, are activated if initiating events (i.e. cause) occur. Hazard scenarios reflect the system behavior to the activated hazards in terms of event propagation from causes to safety consequences, as depicted in Figure 1. The occurrence of events is coupled to observable symptoms in the system. Safety consequences are characterized by their severity.

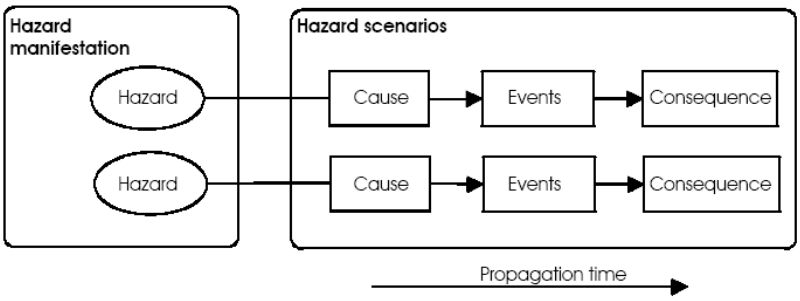

Figure 1 Hazards and hazard scenarios

Different hazard scenarios can originate from the same hazard (hazard tree, Figure 2). Furthermore, different hazard scenarios can lead to the same safety consequence (consequence tree, Figure 3).

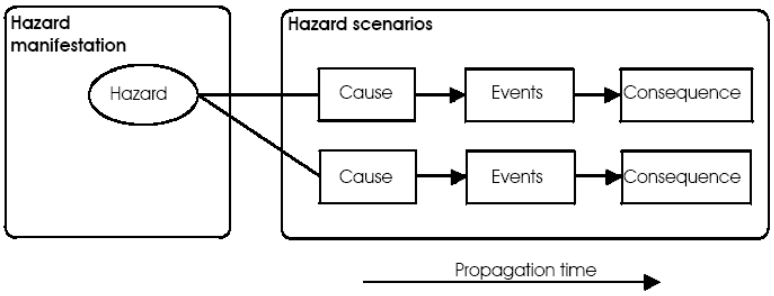

Figure 2 Example of a hazard tree

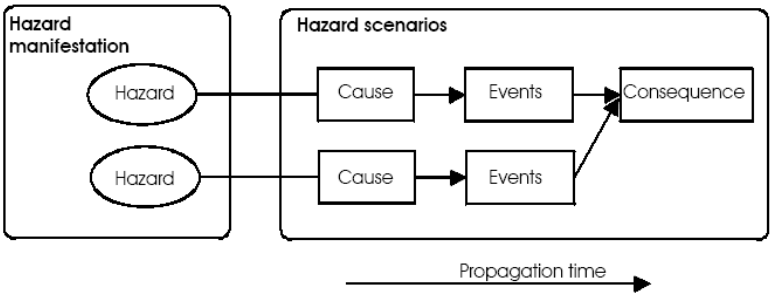

Figure 3 Example of a consequence tree

HAZARD ANALYSIS PROCESS

According to the ECSS-Q-40-02A protocol on hazard analysis, the basic steps comprised in hazard analysis are:

- Step 1: Define the hazard analysis implementation requirements;
- Step 2: Identify and classify the hazards;
- Step 3: Decide and act on the hazards;
- Step 4: Track, communicate and accept the hazards.

Step 3 and 4 are comprised in phase 2 and 3 of the HACCP analysis (CCPs determination and Establishment of a monitoring system). Therefore only the first 2 steps are addressed in phase 1.

Step 1: Define hazard analysis implementation requirements

Task 1: Define the scope of hazard analysis

The hazard analysis procedure for the methanogenesis units is implemented to evaluate the safety and quality control of the methanogenesis units. Methanogenesis is the anaerobic degradation of organic waste on the one hand in an energy rich off-gas (biogas) and on the other hand into a recalcitrant fibre-containing residue. For the identification of the hazards associated with this process, safety requirements concerning human health and the good operation practice and performance of the reactor units are taken into account. A scoring scheme for the severity of safety consequences for the classification of hazard scenarios is presented below.

Table 1 Safety consequence severity categorization

Category	Severity	Severity of safety consequence
I	Catastrophic	Loss of life, life threatening or permanently disabling injury or occupational illness; Irreversible destruction of reactor units
II	Critical	Temporarily disabling, but not life-threatening injury or illness; Major damage to reactor units
III	Marginal	Minor injury, minor disability, minor occupational illness; Minor damage to reactor units
IV	Negligible	Less than minor injury, minor disability, minor occupational illness; Less than minor damage to reactor units

Task 2: Define the system to be analyzed

Methanogenic anaerobic degradation of organic waste is an environmentally attractive way for the conversion of organic waste on the one hand in an energy rich off-gas (biogas) and on the other hand into a recalcitrant fibre-containing residue. As a whole, the anaerobic decomposition of organic waste is generally considered to be a four step process: hydrolysis, fermentation, acetogenesis and methanogenesis. The main aim of the methanogenic unit is to maximize the conversion of organic waste into biogas.

The high-load methanogenic digestor is a 10 L anaerobic reactor, used for the anaerobic digestion of the defined feed. The digester (continuously stirred tank reactor, CSTR) is maintained at a constant temperature of 34°C and is shaken at 70 rpm. The reactor is fed batch wise at regular time intervals. The reactor is fed in quantities of 0.5 L feed/day. The liquid reactor volume is set at 7.5 L (resulting in a hydraulic retention time of 15 days). The solid retention time is 40 d. The biogas passes by an electronic milligascounter device.

The composition of the feed for the high-load methanogenic (2% dry matter) reactor is : 10% Spirulina (95% DM); 24% wheat straw (95% DM); 22.5% fresh cabbage (9% DM); 22.5 % soya (90% DM); 21.5 % faeces (10% DM).

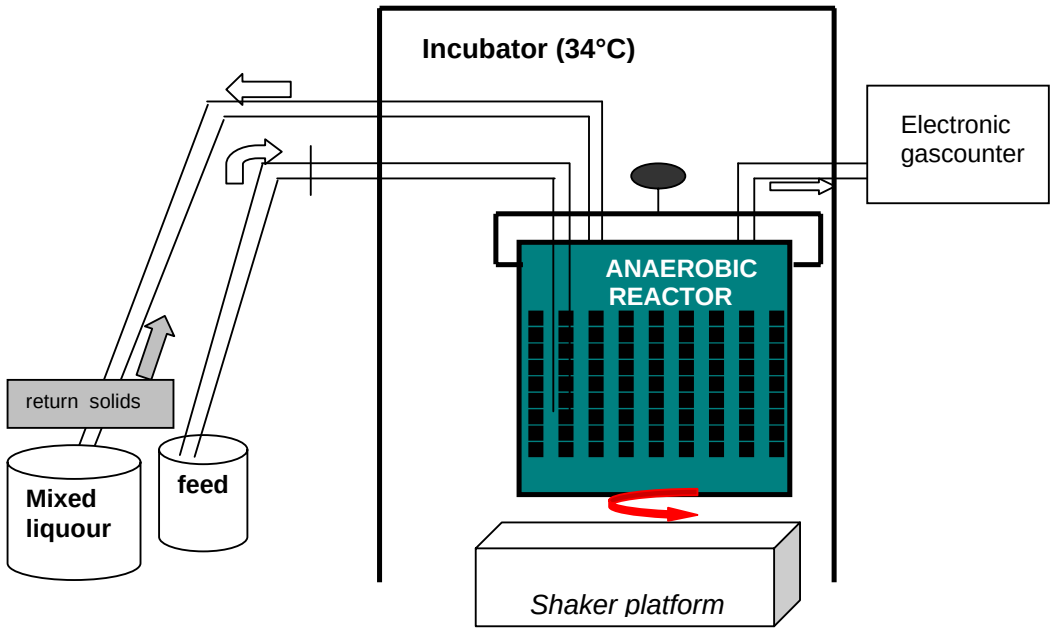

Figure 4 High-load methanogenic reactor

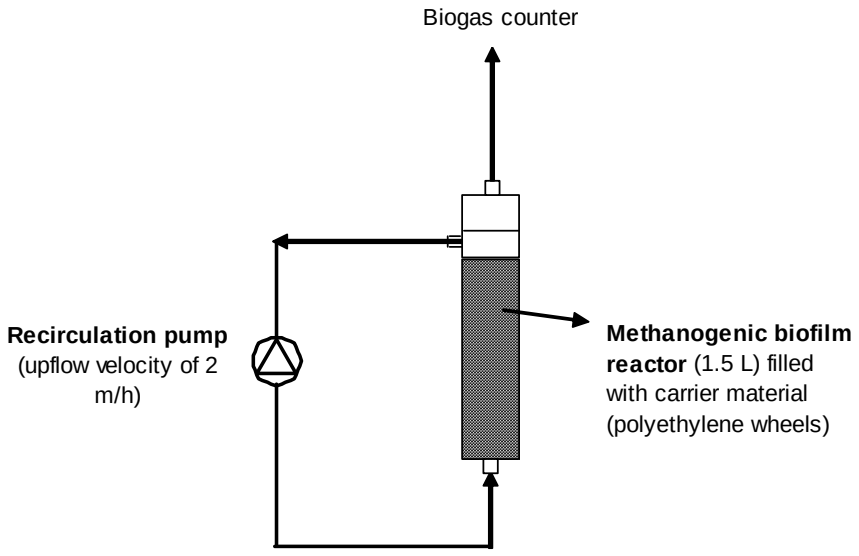

Figure 5 Low-load methanogenic reactor

The low-load methanogenic reactor is a fixed-bed biofilm reactor with a volume of 1 L, filled with 1 dm^3 of polyethylene wheels. This reactor is fed with the returned effluent from the *Fibrobacter* digestion followed by the sub-critical liquefaction is added to the fixed-bed biofilm reactor. The biogas passes by an electronic milligascounter device.

Step 2: Identify and assess the hazards

Task 3: Identify hazard manifestations

The hazards related to the methanogenesis reactor are of biological, chemical and physical nature. These hazards relate to both the process and the users.

Microbiological hazards

In the methanogenic units, microbiological hazards relate to the presence of pathogenic organisms (microbial or viral) that can contaminate compartment IV and thus pose risks to human health. Similarly, toxins and genetic elements (chromosomes, genomes, plasmids, transposons, and vectors) that contain nucleic acid sequences associated with the pathogenicity of microorganisms or that encode for toxins are potential biological hazards that may endanger human health.

Chemical hazards

Certain chemical compounds may lower the reactor performance by causing toxicity to the methanogenic microbial community. Adverse effects can however also be manifested at the level of plant production or human health when noxious compounds are allowed to reach the subsequent compartments in MELISSA.

Physical hazards

Physical hazards of importance to the methanogenic units are related to temperature, pH, anaerobic conditions and liquid or gas leaks which mainly affect the reactor performance. Leaks of liquid or gas are however also of importance for human safety.

The appearances of the hazards are given according to the rules of the 5M i.e. the Material, the Methods, the Manuel labour, the Medium and the raw Material.

Table 2 Hazard matrix

Hazard matrix for methanogenesis unit					
Generic hazards	**Subsystem elements**				
	Media	**Methods**	**Material**	**Raw material**	**Manuel labour**
Microbiological hazard	x	-	x	x	x
Chemical hazard	x	x	x	x	x
Physical hazard	-	x	x	x	x
x = applicable - = not applicable					

Table 3 Hazard manifestation list

Hazard manifestation list	
Subsystem	**Hazard manifestation**
Media	Microbiological and chemical contaminations
Methods	Microbiological and chemical contaminations Acidification of reactor units
Material	Microbiological and chemical contaminations Leak of the medium
Raw material	Contaminations Out-of-date product Acidified feed
Manuel labour	Microbiological and chemical contaminations Acidification of reactor units

Task 4: Identify and classify the hazard scenarios

Table 4 Hazard scenarios

Hazard scenario list for methanogenesis unit				
Hazard manifestation	**Cause – Events – Consequence**	**Consequence severity**	**Observable symptoms**	**Propagation and reaction time**
Microbiological contamination	Presence of pathogens in substrate/materials – Contamination of subsequent MELISSA compartments with pathogens - Illness of crew	Depends on type of pathogen: from Class IV to I	Only detectable after microbial analysis. PCR may be used provided the contaminant is known (i.e specific primers are available)	Ptime & Rtime: Depends on type of pathogen
Chemical contaminations	Presence chemicals in substrate/materials – Inhibition of methanogenesis; contamination of subsequent MELISSA compartments chemicals - Malfunctioning of reactor units ; illness of crew	Depends on type of chemical: from Class IV to I	Important changes in the biogas composition and production rate. Also detectable after chemical analysis.	Ptime & Rtime: Depends on type of contaminant
Leak of the medium	Poor state of materials & disrespect of the protocol- Leakage – Loss of reactor medium	Class II	leakage, loss of volume	Ptime & Rtime dependent on the severity of the leak
Out-of-date product	Conservation date not checked – Change of chemical composition of	Depends on type of product: from	Important changes in the biogas	Ptime & Rtime: Depends on

	product – Undesired products enter the reactor units	Class IV to I	composition and production rate. Also detectable after chemical analysis	type of product
Acidified feed	Storage of the feed for too long – feed becomes acid – pH drop in reactor units	Class II	Important changes in the biogas composition and production rate. Also detectable after measuring pH	Ptime: 2 months Rtime: immediate
Acidification of reactor units	Disrespect of the protocol: adjustment of pH – reactor medium becomes acid – pH drop in reactor units	Class II	Important changes in the biogas composition and production rate. Also detectable after measuring pH	Ptime: dependent on reactor load Rtime: immediate

Phase 2 : Critical control points

CCP DETERMINATION

The second phase in the HACCP analysis it the definition of the CCPs. A CCP is a point at witch control can be applied to prevent or eliminate a safety hazard or reduce it to an acceptable level. CCPs should not be too important in order to focus on a point in particular. It is necessary for each CCP to apply, in complement of the preventive measures defined previously, a control monitoring. A minimum and/or maximum value (critical limit), whose

respect is necessary to ensure the effective control of the CCP, are defined for each parameters. This identification of the critical limits is the first corrective action.
CCPs can be determined based on a decision tree (Figure 6).

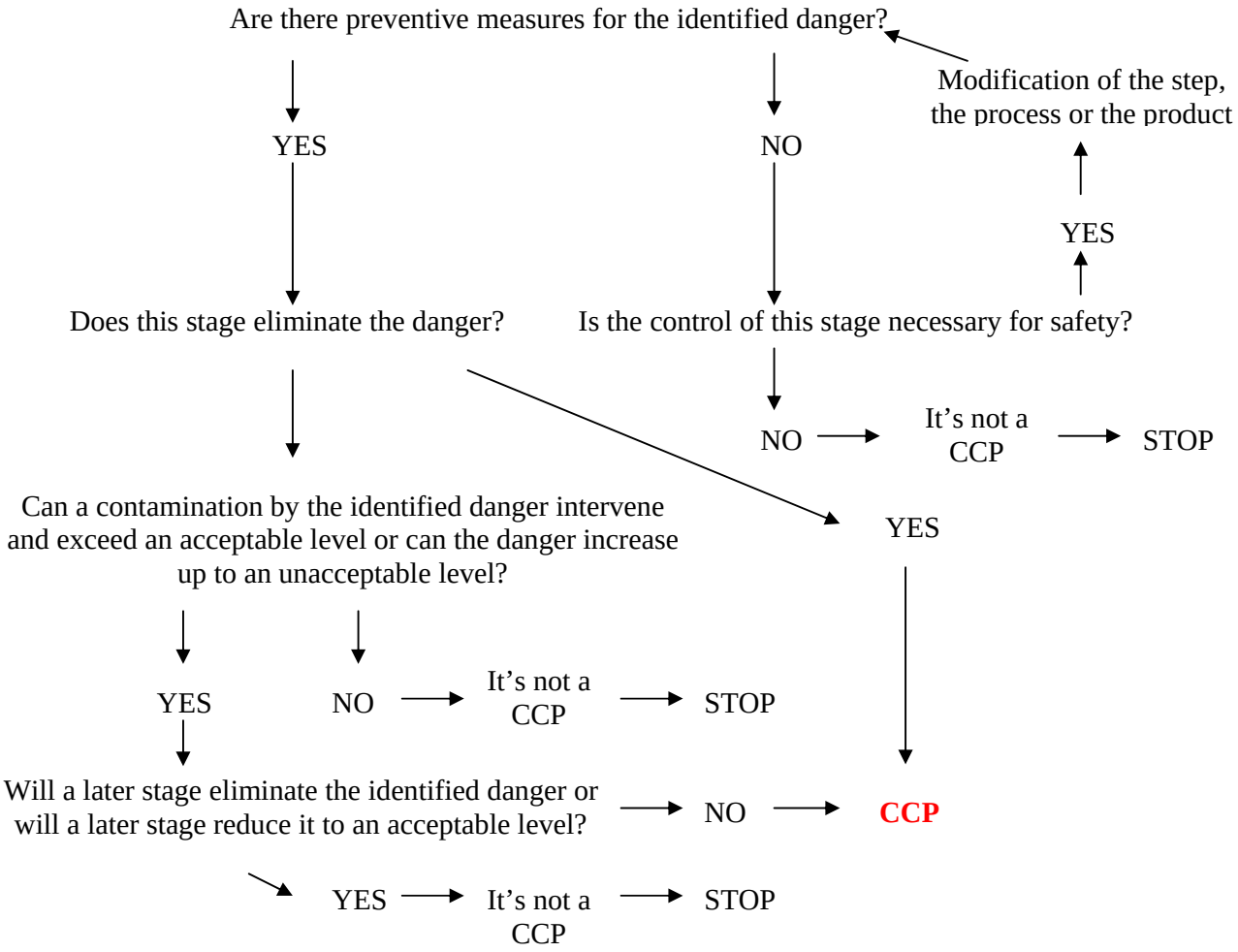

Figure 6 Decision tree for the identification of CCPs
For the methanogenic units following hazards were identified:

Microbiological hazards:

Pathogenic organisms (microbial or viral); toxins and genetic elements associated with the pathogenicity or encoding toxins. The hazard of contamination with pathogenic organisms is controlled by the subcritical liquefaction compartment, which eliminates this potential problem. So far, during the loop experiments, substrate was passed from the high load unit to

the fibrobacter compartment. The effluent of the high load unit is sterilised before being fed to the fibrobacter compartment, which tends to eliminate the problem. Therefore it is not a CCP. As for potentially hazardous genetic elements, no research has been performed on their elimination in the subcritical liquefaction unit. Before assigning a CCP more information is needed regarding the contamination, survival or elimination of these elements. In this respect, reference is made to MELGEN (MELiSSA GENetics) which covers the molecular aspects of the MELiSSA project The general objective of MELGEN is to establish and validate a method and its associated hardware to detect genetic instability and microbial contaminants in the MELISSA compartments.

⇒ CCP1: Elimination of hazardous genetic elements

- Chemical hazards:

Compounds causing toxicity to the methanogenic microbial community, or further MELISSA compartments.The fate of recalcitrant and potentially dangerous chemicals in the subcritical liquefaction compartment needs further investigation. Before assigning a CCP more information is needed regarding the contamination, accumulation and degradation of these compounds. In this respect, reference is made to BELISSIMA. This project investigates the fate of recalcitrant chemicals like endocrine disruptors and pharmaceuticals in the different compartments of MELiSSA. In the MAP study, the usage of Hydrothermolysis under oxidative conditions has shown great value to ensure complete sanitation and liquefaction. However, partial oxidation of the influx results in many uncontrolled chemical reactions, giving rise to a broad range of organic chemicals. It is clear that the reaction conditions for Hydrothermolysis need strict control and follow up to avoid chemical contamination in subsequent units via this way.

⇒ CCP2: Degradation of recalcitrant chemical compounds

- Physical hazards: Temperature, pH, anaerobic conditions and liquid or gas leaks. These parameters are controlled at the level of the methanogenesis units.

⇒ CCP3: Methanogenic process parameters

DETERMINATION OF CCPS CRITICAL LIMITS

The respect of the critical limits insures the control of the CCP (Table 6).

Table 5 Critical limits

N° CCP	Step	Critical limits
1	Elimination of hazardous genetic elements	Limits of molecular tests
2	Degradation of recalcitrant chemical compounds	Limits of chemical analysis
3	Methanogenic process parameters	Temperature = 34 7.2< pH < 7.3 Airtight sealing No leakage

Phase 3: Establishment of a monitoring system

A monitoring system and a control system of the process are established in order to ensure a continuous monitoring. The monitoring system must contain a corrective action plan, documentation and a validation of the HACCP plan. There are procedures to follow when a deviation occurs and actions to confirm that the system according to the plan. These actions must be planned for each CCP, in order to allow an immediate action and a fast elimination of the hazard.

DEFINITION OF THE CONTROL MONITORING FOR EACH CCP

A detailed description of the monitoring of the CCPs is depicted in appendices 1-3.

ESTABLISHMENT OF THE CORRECTIVES ACTIONS

Table 6 Corrective actions

N° CCP	Step	Corrective actions
1	Elimination of hazardous genetic elements	Pasteurisation of the feed (once the reactor works stabile) Treat with subcritical hydrothermolysis (considered in this case as a continuous treatment device)
2	Degradation of recalcitrant chemical compounds	Treat with subcritical hydrothermolysis (stringent control of the reaction parameters to avoid partial oxidation by-products)
3	Methanogenic process parameters	Calibration of the rector set values (Temperature, pH, air tight) Correct if needed

Appendix 1: Elimination of hazardous genetic elements

Objective
The purpose of this instruction is to give the precautions to eliminate hazardous genetic element.

Who?
Competent and authorized personnel.

Where?
Molecular laboratory.

When?
According to a planning elaborated and recorded by the person in charge for the methanogenesis units.

How?

Molecular analysis for the detection of hazardous genetic elements using techniques developed within the frame of the MELGEN activity.
Once potential hazardous elements are identified, the correct molecular technologies can be adapted to detect their presence. As in most molecular biological technologies, it is difficult to single out unknown genetic elements and to quantify them.

Appendix 2: Degradation of recalcitrant chemical compounds

Objective
The purpose of this instruction is to give the precautions for the degradation of recalcitranct chemical compounds.

Who?
Competent and authorized personnel.

Where?
Laboratory with GC and HPLC equipment

When?
According to a planning elaborated and recorded by the person in charge for the methanogenesis units.

How?
GC or HPLC analysis for the detection of recalcitrant chemical compounds.

Appendix 3: Methanogenic process parameters

Objective
The purpose of this instruction is to give the precautions for the methanogenic process parameters.

Who?
Competent and authorized personnel.

Where?
Location of methanogenesis units.

When?
According to a planning elaborated and recorded by the person in charge for the methanogenesis units.

How?
Check temperature settings of the incubator of the reactor. Adjust if necessary.
Measure pH. Adjust if necessary. Regular maintenance and calibration of the analytic equipment used in the follow-up of the methanogenic process parameters is essential.
Check for leakages (visual for liquid, gas leak detector for gas). Repair if necessary.

ESTABLISHMENT OF A DOCUMENTARY SYSTEM

The monitoring and control system are constituted of the 4 types of documents:

- Specification of the system to be analysed. This documentation can be found in the part of hazard analysis.
- Procedures: these documents aim to describe the protocols used to achieve the objectives,
- Instructions: these documents aim to explain the preventive and corrective actions (appendices 1-3),
- Recording supports: With these documents it is possible to keep a copy of the work performed and the results obtained.

CHECK THE SYSTEM AND STAFF TRAINING

HACCP plan is re-examined every year. Moreover, it is evaluated and if necessary adjusted in the course of year to update the new internal requirements (modifications of the raw materials...) and external requirements (environment, customers...).

Staff training is essential and necessary. This training is intended for all people who work with the methanogenesis units. The aim of this training is to inform the personnel on the control of the critical points and to point out the potential risks and their consequences on human health and process stability.

3 PREPARATION OF METHANOGENESIS UNITS FOR ASSEMBLY

Substrate composition and preparation of residue

The composition of the 2% DM substrate was similar to the previous TN's:

10% DM *Spirulina* (95% DM): 2.85 g/L
24% wheat straw (95% DM): 6.65 g/L
22.5% fresh cabbage (9% DM): 6.3 g/L
22.5 % soya (90% DM): 6.3 g/L
21.5 % faeces (10% DM): 6 g/L

After CSTR fermentation, the solids of the digested effluent were separated from the liquid matrix by centrifugation (5 min. at 7000 rpm). Solids were collected in a closed vessel, frozen and subsequently distributed to Partner 2 (about 400 g DM solids) and to Partner 4 (about 100 g DM solids).

Experimental set-up of the high-load methanogenesis unit for assembly

A 10 Liter glass anaerobic reactor is used for the anaerobic digestion of the defined feed. As indicated in Figure 7 the digester is maintained at a constant temperature of 34°C by placing it in an incubator. The reactor is a CSTR-type (continuously stirred tank reactor) and is shaken on a shaker platform (INNOVA shaker) at a constant 70 rpm.
The reactor is fed batch wise at regular time intervals. For each volume of the feed fed to the reactor, a same volume of stirred mixed liquor is withdrawn simultaneously. The biogas passes by an electronic milligascounter device (Fachhochschule Bergedorf, Hamburg-Harburg, Germany).
The volumetric loading rate of the mesophilic digester was held at 1.4 g COD/L.day (Chemical Oxygen Demand) over a period of 3 months in order to obtain the necessary amount of fibrous residue (about 400 g DM (Dry Matter) to distribute to Partner 2 and about 100 g DM to Partner 4). Reactor performance was stable at the given volumetric loading rate.
The reactor was fed in quantities of 0.5 L feed/day. In order to maintain a hydraulic retention time of at least 15 days, the liquid reactor volume was set at 7.5 L. To ensure a solid retention time of 40 d (postulated as the desired SRT in TN 1.7) solids represented in 0.313 L of the daily removed 0.5 L mixed liquor were collected by centrifugation (5 min. at 7000 rpm) and returned to the methanogenic reactor.

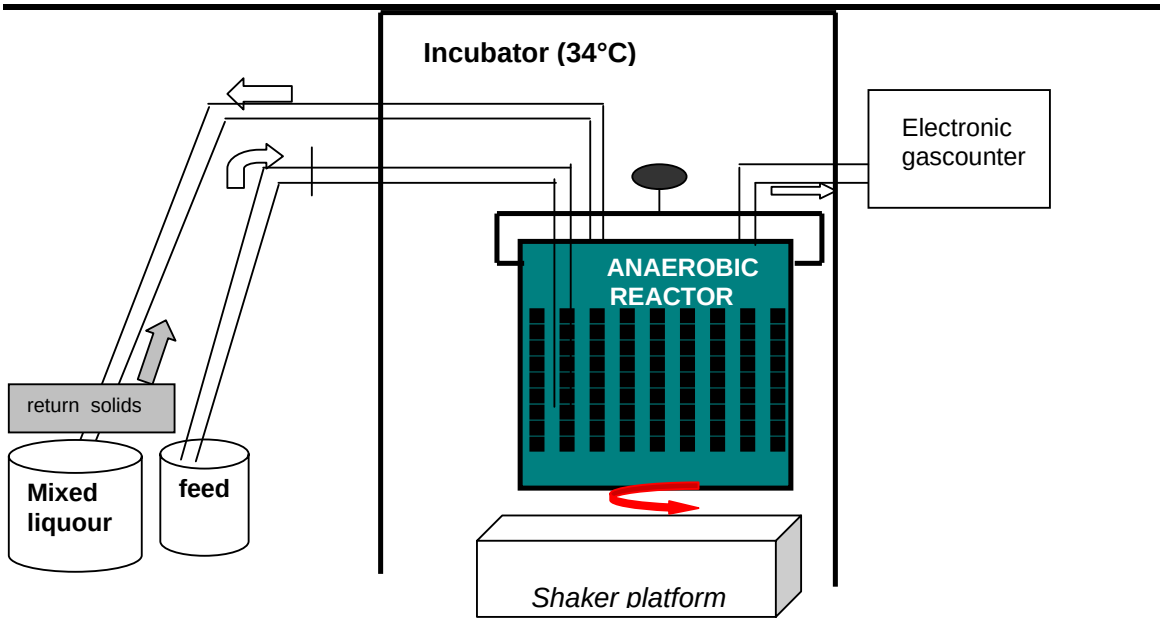

Figure 7 Scheme of the methanogenic digester.

Experimental set-up of the low-load methanogenesis unit for assembly

The same reactor set-up as described in the previous Technical Note (TN 1.7) was used. The fixed-bed biofilm reactor had a volume of 1 L. The reactor was filled with 1 dm^3 of polyethylene wheels (Kaldnes). To initiate the biofilm formation, 700 mL of tap water and 300 mL of sludge from the CSTR were added. Subsequently the liquid was continuously recycled at an up flow velocity of 2 m/h and on a daily basis between 0.5 and 1 g COD/L.d was dosed during a period of 8 weeks at mesophilic temperature ranges. Subsequently, the excess of (free) sludge was removed from the reactor and 1 L of the returned effluent from the *Fibrobacter* digestion followed by the sub-critical liquefaction was added to the fixed-bed biofilm reactor. The biogas passes by an electronic milligascounter device (Fachhochschule Bergedorf, Hamburg-Harburg, Germany). A schematic presentation of the reactor set-up is depicted in Figure 8.

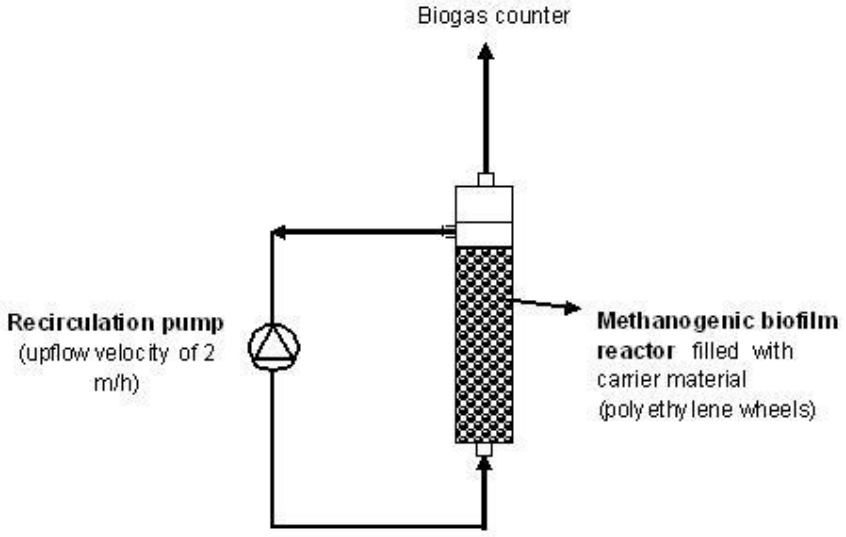

Figure 8: Schematic presentation of the reactor set-up of the low-load methanogenesis unit.

Influent and effluent analysis of high-load methanogenesis unit

VFA-analysis (Volatile Fatty Acid), DM-content (dry matter), VS-content (volatile solids), COD (chemical oxygen demand), Kjeldahl-N (KjN), Total Ammonium-N (TAN), and Total Oxidized-N (TON) were measured prior to and after fermentation.

The amount of biogas was monitored continuously with an electronic gas counter and the biogas composition was determined by means of gas chromatography (GC-TCD).

CHARACTERISATION OF THE INFLUENT

Table 7 Feed characterisation

DM-content	COD	TAN	Kj-N	VS	ash-content
2.8%	21 g/L	0.41 g/L	1.2 g/L	24 g/l	4.4 g/l

CHARACTERISATION OF THE MIXED LIQUOR IN THE REACTOR

The dry matter (DM) content profile of the mixed liquor in the digestor is shown in Figure 9. Although in the start-up phase there was a built-up of DM, the value reached a plateau after 20 days.

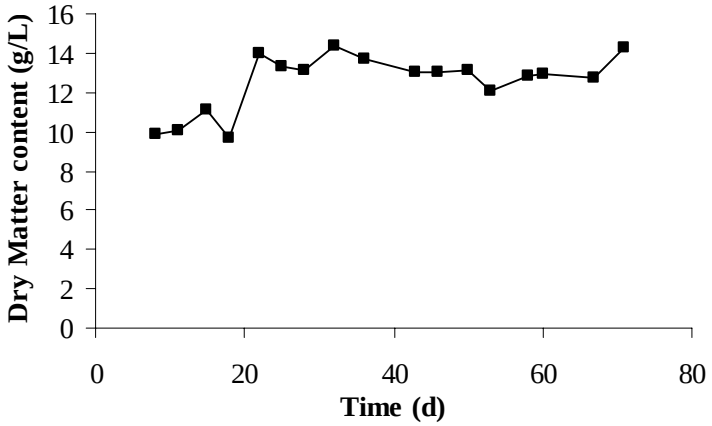

Figure 9 Dry matter profile of the mixed liquor in the digestor

Due to the higher solid retention time, DM content increased compared to values obtained for TN 1.7. However, equilibrium was reached after a start-up period of 20 days.

BIOGAS PRODUCTION

During the fermentation of the raw substrate, the biogas production was constantly monitored with an electronic gas counter (Figure 10).

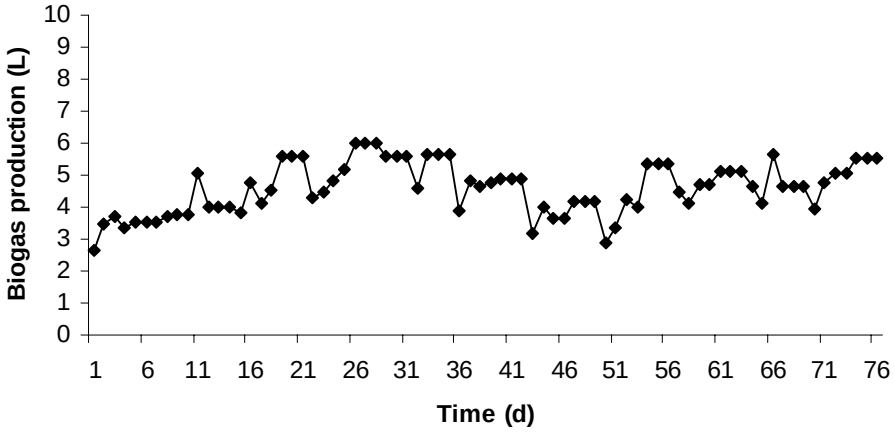

Figure 10 Daily biogas production of the high load methanogenic digestor

On average 4.6 ± 0.8 L of biogas was produced per day. This production was found to be in accordance with the volumetric loading rate with on average a production of 0.44 L biogas/g COD or a biogas yield of 87.7%. The average methane content, measured over a 2 months period, accounted for 62.8 ± 1.8% of the total biogas production.

CHARACTERISATION OF THE EFFLUENT

In first instance, standard analysis was performed on the reactor effluent. Average results are shown in Table 8.

Table 8 Effluent characterization

DM-content	COD	TAN	Kj-N	VS	ash-content
0.47%	6.9 g/L	0.71 g/L	1.0 g/L	3.6 g/l	1.1 g/l

COD profile

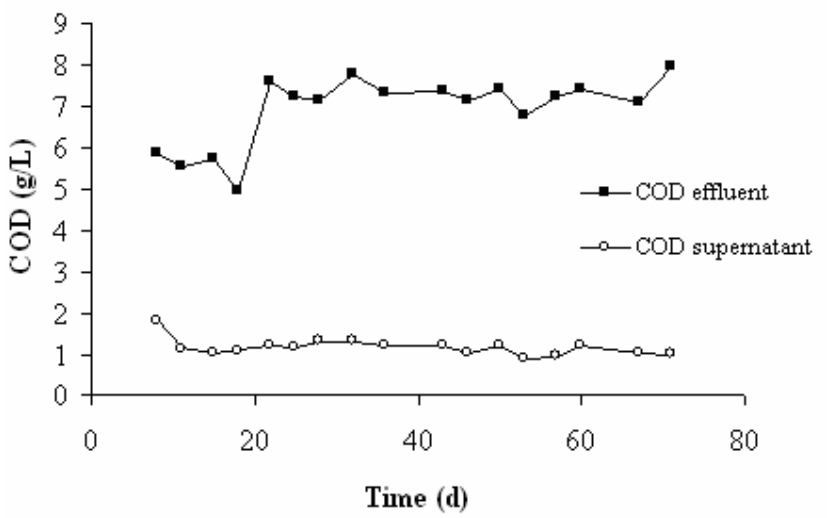

Figure 11 Profile of the COD of the effluent and its supernatant

As can be seen from Figure 11, the COD of the reactor effluent reaches a stable value of about 7 g/L after a start-up period of 20 days. Only 1 g/L is due to the COD content of the supernatant of this effluent, which indicates that most COD is comprised in the biomass and fibrous particulates leaving the reactor.

Nitrogen profile

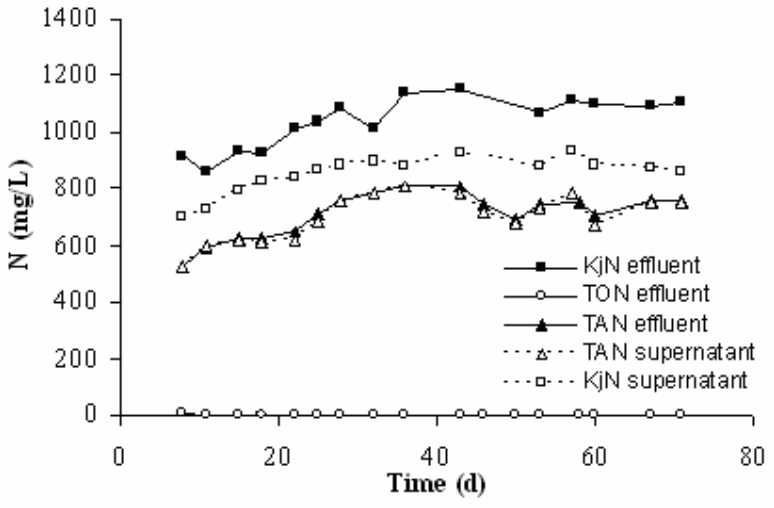

Figure 12 Profile of the KjN, TAN and TON of the effluent and its supernatant

From Figure 12, it is clear that the organic bound nitrogen is converted during anaerobic digestion into solubilized ammonia. There is no oxidized nitrogen present (TON level is zero). All ammonia of the effluent is in the soluble phase (supernatant) since the TAN-level of the supernatant was found to be similar to the TAN-level of the total effluent. Some nitrogen is still organic bound (part of KjN not attributed to TAN or TON), comprised in the biomass and fibrous particulates leaving the reactor.

Volatile Fatty Acids

Volatile fatty acids where extracted from the digester effluent with diethylether and analyzed with GC-FID (with internal standard). The following fatty acids have been determined: acetic acid (2.7 mg/L), propionic acid (0.5 mg/L), and traces of isobutyric acid, isovaleric acid and iso capric acid. It can be concluded that all VFA-concentrations were found to be very low (< 5 mg/L). This clearly indicates the high organic carbon removal and the stability of the digester.

4 LOOP EXPERIMENT

Influent and effluent analysis of low-load methanogenesis unit (6th closed loop experiment)

After the start-up period of the fixed-bed biofilm reactor, returned effluent from the *Fibrobacter* digestion followed by the sub-critical liquefaction was added to the fixed-bed biofilm reactor. The liquids were continuously recirculated with an up flow velocity of 2 m/h. The biogas production and parameters as COD, VFA and pH were followed on regularly basis, during a total period of 7 days.

CHARACTERISATION OF THE INFLUENT

The returned effluent of the near-critical liquefaction test, received from Partner 4, (i.e. the influent for the biofilm reactor) was analysed for pH, COD, Kj N, TAN, TON and VFA. The characteristics are presented in Table 9.

1 L of sample (received from Partner 4) was added to the low-load methanogenic reactor unit. The volumetric loading rate at time 0 was 0.23 g COD/L.d. Prior to the experiment, the pH of the effluent was adjusted to 7.5 with $NaHCO_3$.

Table 9 Characteristics of the sample received from Partner 4 and treated by anaerobic digestion (solid digester residue) followed by the *Fibrobacter* unit (Partner 2) and near-critical liquefaction unit (Partner 4)

Parameter	Unit	Sample
pH	-	6.68
CODt	mg/L	223
CODs	mg/L	244
Kj-N	mg/L	71.5
TAN	mg/L	58.5
TON	mg/L	0
VFA	mg/L	167
acetic acid	mg/L	164
propionic acid	mg/L	3
sobutyric acid	mg/L	0
butyric acid	mg/L	0
isovaleric acid	mg/L	0
valeric acid	mg/L	0
isocapronic acid	mg/L	0
capronic acid	mg/L	0

As can be seen from Table 9, the sample had a COD value of about 223 mg /L. Since there were virtually no solids left in the effluent after treatment by Partner 4, all COD was present in the soluble phase. These COD values are low in comparison with the subcritical sample

received and described in Technical Note 1.7 (Table 6, 2.7 g CODt/L and 1.1 g CODs/L). This is due to the different liquefaction treatment was applied (Near-critical oxidation with H_2O_2). The volatile fatty acids concentration was 170 mg/L.

CHARACTERISATION OF THE EFFLUENT

For a period of 7 days, the liquid was recycled over the fixed-bed biofilm reactor with an upstream velocity of 2 m/h. Parameters such as pH, COD, VFA, Kjeldahl nitrogen, TAN, TON and biogas production were measured regularly. Since the effluent received form Partner 4 contained virtually no solids, and all COD was in the soluble phase, only $COD_{soluble}$ was monitored during the experiment. This excludes the possible bias of measuring COD originating form material released by the biofilm. The results are shown in Table 10.

Table 10 Results of the fixed-bed biofilm reactor experiment with effluent of the near-critical liquefaction unit.

Time (d)	0	2	5	7
pH	7.5	7.6	7.6	7.4
CODs (mg/L)	243.9	84.3	102.6	101.9
VFA (mg/L)	167	10*	5**	63***
Kj N (mg N/L)	71.5	98.9	99.0	98.7
TAN (mg N/L)	58.5	69.6	68.8	71.3
TON (mg N/L)	2.5	0	0	0
Cumulative biogas production (mL)	0	40	60	60

* 5 mg/L acetic acid, 1 mg/L propionic acid, 1 mg/L isocapronic acid and 3 mg/L capronic acid
** 4 mg/L acetic acid and 1 mg/L capronic acid
*** 45 mg/L acetic acid, 14 mg/L propionic acid, 3 mg/L butyric acid and 1 mg/L isovaleric acid

After one day, a biogas volume of 40 mL was measured. Hence, there was no inhibition in biogas production. After 7 days of recirculation, the COD soluble decreased from 244 to 102 m/L, i.e. a 58 % decrease.

The Kjeldahl nitrogen remained constant during the experiment, although the measured values were higher than the initial concentration. This can be explained by Kj-N originating form the biofilm of the reactor. The TAN concentration increased slightly and the TON concentration

was negligible. Overall, it can be concluded that the nitrogen compounds did not change significantly during the anaerobic digestion in the fixed-bed biofilm reactor.

BIOGAS PRODUCTION

The theoretical biogas production, considering the fact that 1 g COD removal results in 0.5 L biogas with a biogas composition of 70 % CH_4 and 30 % CO_2, was 115 mL. The measured biogas volume was 60 mL. This value is underestimated because of several reasons. Firstly, an overpressure in the reactor is needed before the biogas can be transferred to the gas column. Secondly, the biogas produced is partly entrapped within the polyethylene wheels. Most of the biogas can be released from the rings by manually shaking the reactor but some gas bubbles remain entrapped in the matrix of rings. Overall, it can be concluded that about 52 % of the COD present in the effluent of the liquefaction unit was converted into biogas. This value is comparable to the results described in TN 1.4 (41 % COD removal after 4 days), TN 1.6 (41 % removal after 5 days) and TN 1.7 (50% after 7 days). The composition of the biogas was determined after 1 day and at the end of the test. The results are presented in Table 11.

Table 11 Biogas composition after 1 day of recirculation and at the end of the test

	t = 1 d	t = 7 d
CH_4 (%)	59 ± 0.8	56 ± 1.1
CO_2 (%)	41 ± 0.8	44 ± 1.1

5 CONCLUSIONS AND FUTURE PERSPECTIVES

In this technical note, HACCP analysis of the methanogenesis units was performed following the hazard analysis protocol, based on the ECSS-Q-40-02A report on Space Product Assurance - Hazard analysis. Critical control points (CCPs) and their critical limits were determined and a monitoring system was established.

Further the methanogenesis reactor units were made ready for assembly with the hydrothermolysis reaction unit to form the Total Conversion-unit (TC –unit).

The high-load methanogenic reaction unit was operated at the conditions that were proposed in TN 1.6. The operation at a solid retention time of 40 days, showed stable performance with a conversion of COD into biogas of over 87%.

The fermentation of the returned effluent treated by Partner 1 (anaerobic digestion), Partner 2 (Fibrobacter) and Partner 4 (near-critical liquefaction) in a fixed-bed methanogenic biofilm reactor resulted in a 52 % CODs removal after 7 days of recirculation. The nitrogen compounds did not change significantly during the experiment.

For WP 1.9, the two methanogenis reaction units will be assembled with the hydrothermolysis unit to form a Total Conversion unit (TC-unit). Biosafety efficiency of this TC-unit will be determined and HACCP analysis of C1 of MELiSSA will be compared with the TC-unit. Finally, the methane conversion into SCP and carbon dioxide will be calculated.