M E L i S S A

T E C H N I C A L N O T E

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TECHNICAL NOTE: 1.5

EVALUATION OF THERMAL TREATMENT PROCEDURES FOR STERILISATION

(PROJECT: A TOTAL CONVERING AND BIOSAFE LIQUEFACTION COMPARTMENT FOR MELISSA)

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1 INTRODUCTION AND OBJECTIVES

The main objectives of the first technical note of the second phase of this project were on the application and demonstration of additional technologies to further enhance the biogas yield of the CSTR (continuously stirred tank reactor) and to assure a complete sanitized effluent after CSTR-treatment. The tasks described for this TN are given below:

INPUTS

- Batch methanogenesis units
- Latest test results with the methanogenesis reactor
- Required test equipment for the characterisation of the solubles released during sterilisation and fermentation (DTU)
- Pilot-scale reactors for sterilisation tests (DTU)

Tasks included

- Sterilisation tests with thermal treatment pilot-scale reactors
- Detailed characterisation of solubles released during hydrolysis
- Batch methanogenesis tests with solubilised products
- Distribution of methanogenic culture to Partner 3
- Collect information with regard to HACCP about biosafety

After a research stay of 6 months in between the two phases of the project, it was decided to explore a newly developed alkaline wet oxidation (AWO) technology for the complete sanitation of the CSTR effluent and to evaluate how much the biogas potential in the CSTR-step can be increased by performing a second digestion after AWO treatment. Apart from these results, the company who invented the WR-treatment (alkaline wet oxidation) to treat BSE-contaminated waste was identified. Finally, according to the last progress meeting at DTU

(Denmark), a chemical is proposed which is ideally suited to perform and set up the HACCP-protocol.

In Figure 1, the conceptual scheme of a total converting liquefaction compartment as designed and agreed upon in the latest progress meeting is depicted. The concept combines three technologies being methanogenesis, *Fibrobacter* liquefaction and thermal sub-critical liquefaction.

The sterilization step indicated in the methanogenesis unit 1 was the focus of this research. In order to provide complete biosafety in the system according to the HACCP standards, a sterilization treatment is needed that can completely kill off prion-like material. Furthermore, the sterilization should preferentially also enhance the biodegradability of the digested solids from the CSTR, thereby enhancing the total methane yield and decreasing the required volume of the reactors in the methanogenesis units.

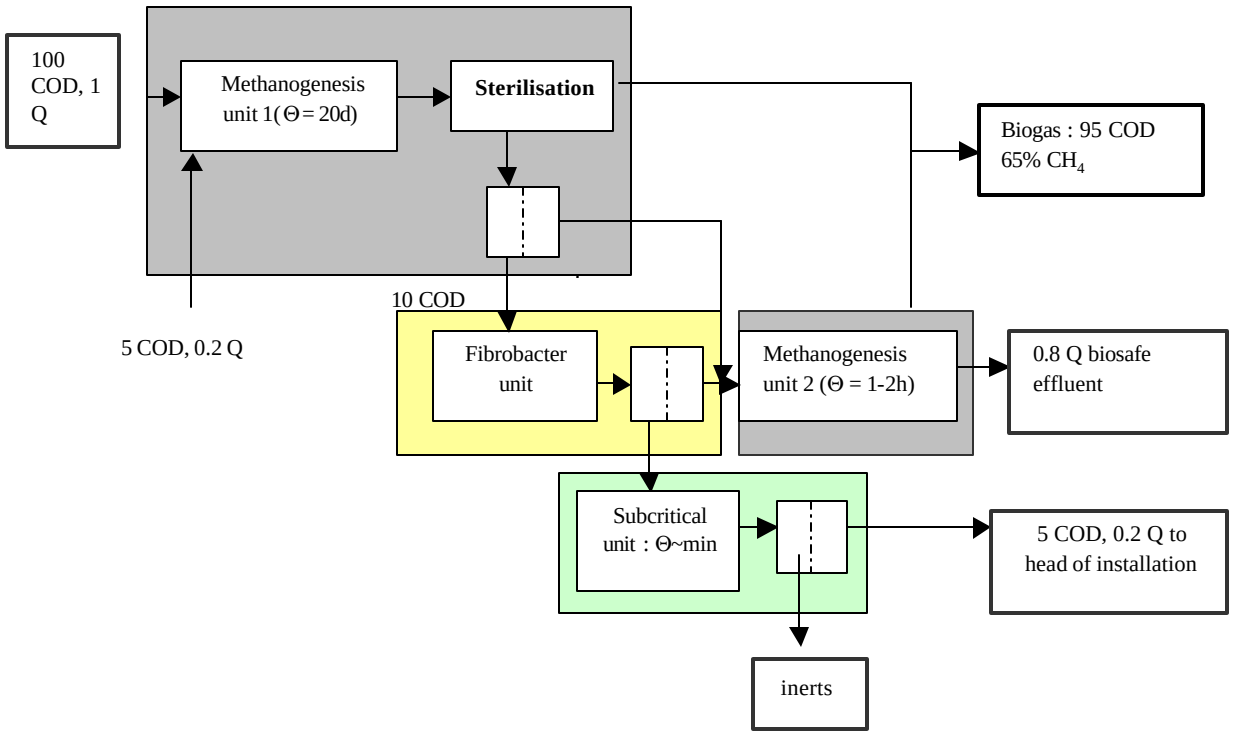

Figure 1: Conceptual scheme of a total converting and biosafe liquefaction compartment for MELiSSA

2 MATERIALS AND METHODS

Experimental set-up of the high-load methanogenesis unit

MESOPHILIC DIGESTER

A 10 Liter anaerobic PVC-reactor is used for the anaerobic digestion of the defined feed. As indicated in Figure 2, 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 two minutes/hour on a shaker platform (INNOVA shaker) at a constant 90 rpm.

The reactor was operated fed-batch wise at regular time intervals. For each volume of the feed fed to the reactor, a same volume of stirred mixed liquour is withdrawn simultaneously. The biogas passes by an electronic milligascounter device (Fachhochschule Bergedorf, Hamburg-Harburg, Germany) with a resolution of 1 ml and an accuracy of 3%. The biogas composition has been monitored during the preparation and fermentation of the batch fibrous residues.

The volumetric loading rate of the mesophilic digester was held at 2.17 g COD/L.day (Chemical Oxygen Demand) over a period of 3-4 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 dry matter content of the synthetic feed was kept at 2% dry matter. 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 of both reactors was set at 7.5 L.

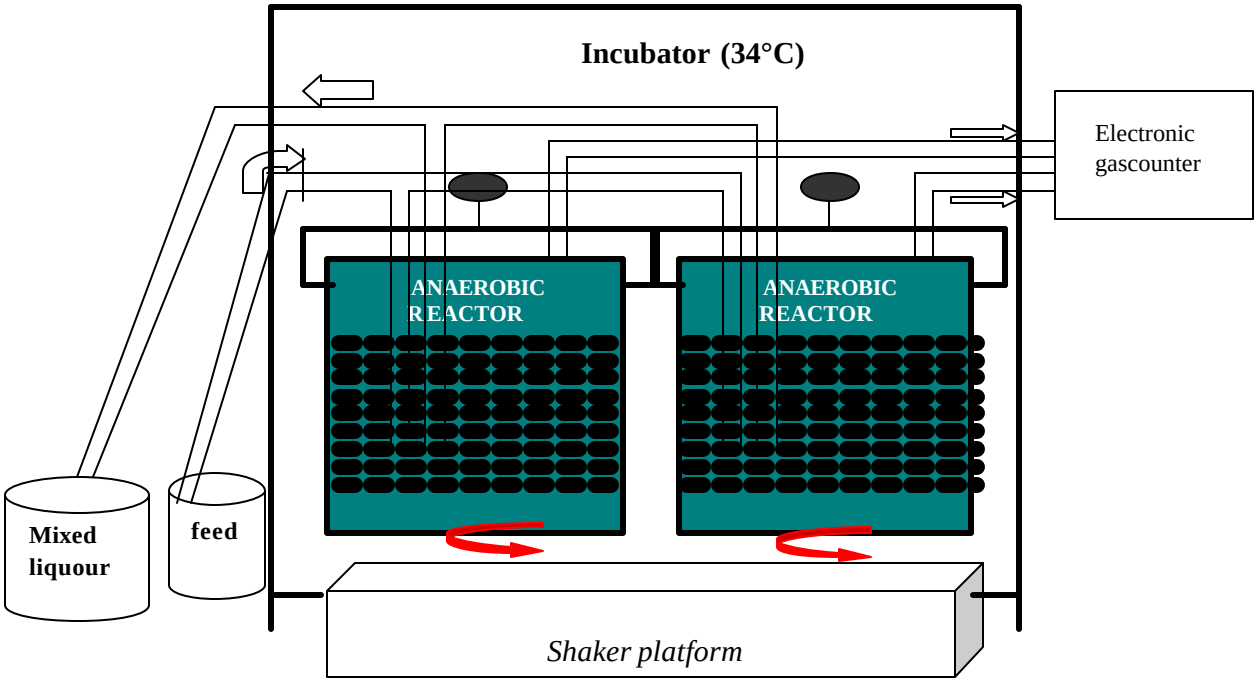

Figure 2: Scheme of the 2 mesophilic digesters for the fermentation and subsequent distribution of the digester residues (approximately 400 + 100 gram DM)

ALKALINE WET OXIDATION TREATMENT OF THE CSTR EFFLUENT

The digested waste from the CSTR was oxidized under 3 different conditions (referred to as A-C). AWO experiments with digested biowaste were performed batch-wise (duplicate) by using 0.5 liter of CSTR-effluent for each experiment.

The AWO conditions applied on the digested biowaste (A-C) are summarized in Table 1. The AWO reaction time was set at 15 min for all experiments. The pH of the solutions was measured before and after wet oxidation (Table 1).

The oxidation effect during AWO was obtained by adding a determined amount of hydrogen peroxide (H_2O_2) to the solution to be treated. AWO condition A represented a hydrothermal treatment, whereby no hydrogen peroxide was added. For AWO conditions B and C, an amount of hydrogen peroxide (6 respectively 12 ml) was added to the effluents corresponding to about 15% and 25-30% oxidation of the VS content of the materials, respectively. This

concentration range was previously found (Lissens et al., 2004) to be optimal to maximally enhance the biogas potential from organic household waste. Apart from the bicarbonate already present in the digester effluent (pH = 8.3), no experiments were performed involving the addition of Na_2CO_3 (Table 6.1).

Table 6.1. Alkaline Wet oxidation (WO) conditions for digested effluent from the CSTR (Condition A-C)

Parameter WO conditions	**Digested effluent from CSTR**		
	A	**B**	**C**
Temperature (°C)	170	170	170
Time (min)	15	15	15
ml H_2O_2 added	0	6	12
Na_2CO_3 (g/L)	0	0	0
pH before WO	8.3	8.3	8.3
pH after WO	10.03	9.37	9.28
Total Solids (g/L) after AWO	13.88	20.52	16.78
Volatile Solids (g/L) after AWO	9.31	14.24	11.90

WO experiments were carried out in a high-pressure autoclave at the DTU. The autoclave was designed as a cylindrical vessel (V = 1890 ml) made of Sandvik Sanicro 28 (27% Cr, 31% Ni, 3.5% Mo, 1% Cu). The wet oxidation procedure consisted of following steps:

- The solution was first heated to 100 degrees C and the atmospheric air was released by opening the pressure valve. This venting out was repeated a few times.
- Next, the solution was further heated to a temperature of 170 degrees C. For all AWO conditions, the solution was held at this temperature for a period of 10-15 minutes.
- After this holding time, H_2O_2 was added (for AWO condition B and C only) and the pressure was increased to 12 bars and further on monitored in function of time. The oxidation reaction caused by the H_2O_2 caused a temporary pressure increase. After the

increase in pressure stabilized (appr. 5 minutes), the oxidation reaction was terminated by decreasing the pressure to 11 bars.

- Immediately after, the solution at a pressure of 11 bars was flushed into an empty vessel by sudden release of the pressure (steam explosion).

BATCH FERMENTATION TESTS AFTER AWO TREATMENT

Batch fermentation tests were set up with effluent (mixed liquour) from the CSTR reactor that had been treated with the AWO (alkaline wet oxidation) process at the Technical University of Denmark (Partner 5). Anaerobic sludge (mixed liquour) of the main methanogenic digesters was used as an inoculum for all fermentation tests. All experiments were performed in 500 mL erlenmeyers containing a fixed amount of mixed liquour from the two main mesophilic reactors. The volume of mixed liquour present in each batch bottle was 200-300 mL. The mixed liquour contained a solid phase, existing both of flocculated non-granular sludge (methanogenic bacteria) and residual fibers from previous fermentations. The liquid phase consists mainly of soluble biopolymers. The experiments were run over a period of 12 days by adding wet oxidized effluent at regular time intervals. For every batch test, the amount of substrate added corresponded to 1 g VS (volatile solids), corresponding to a maximum achievable methane production of 350 mL.

Bottle 1 and 2 contained duplicate samples of the wet oxidized waste of AWO condition A, bottle 3, 4 and 5 the triplicate samples of AWO condition B and bottle 6, 7 and 8 the triplicate samples of AWO condition C. Bottle 9 and 10 contained the control samples, namely the untreated digested CSTR effluent. The volume of biogas and pH was continuously measured for each bottle. All fermentation trials were performed in duplicate or triplicate to check for the reproducibility.

Bioconversion efficiencies were calculated based on the general assumption that 1 g of VS (volatile solids) can be transformed in 0.5 L of biogas. This corresponds to the theoretically maximum production of 0.35 L CH_4/g glucose (Verstraete et al., 1996).

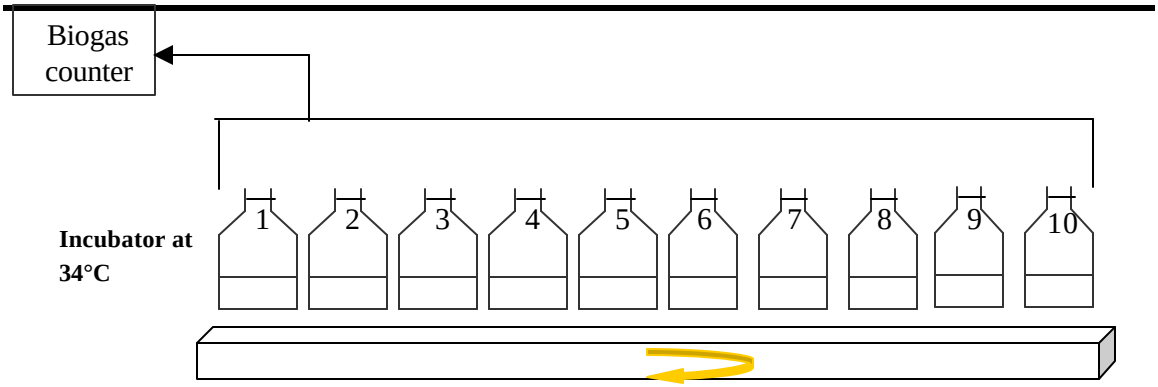

Figure 3: Experimental set-up for batch fermentation tests with untreated digester effluent and wet oxidized digester effluent

Substrate composition and preparation of residue (4th closed loop)

SUBSTRATE COMPOSITION

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 decantation, collected in a closed vessel, frozen and subsequently distributed to Partner 2 (about 350 g DM solids) and to Partner 4 (about 100 g DM solids).

3 RESULTS AND DISCUSSION

AWO treatment of the digested CSTR effluent

Effluent of the CSTR reactor that was treated with the AWO process under different conditions (A-C) was subjected to a second anaerobic digestion. In Figure 4, the biogas yields for the different treatments (Control, AWO conditions A-C) for 1 feeding cycle are depicted.

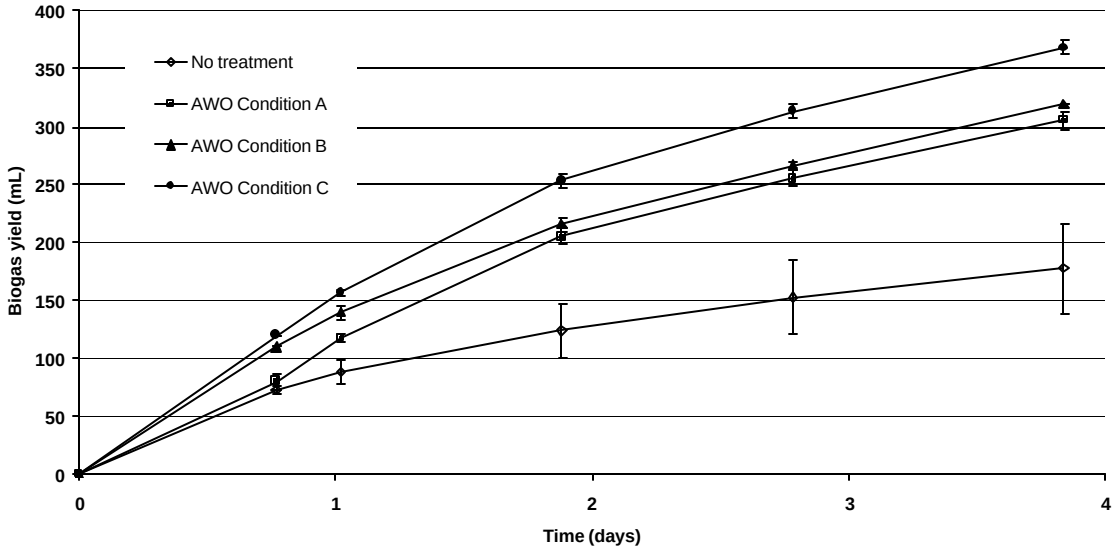

Figure 4: Graph of the biogas yields (mL) for the different treatments (Control, AWO condition A-C) for 1 feeding (1 g VS)

From Figure 4, it can be seen that the AWO treatment (Conditions A, B and C) caused an increase of the biogas yield of the effluent compared to the untreated digested CSTR effluent. The highest biogas yield was achieved when the effluent of the CSTR digester was subjected to the AWO process with conditions C (approximate 110% increase of the methane yield compared to the untreated sample). A treatment with the AWO process under conditions A and B gave similar biogas yields (approximately 80% increase compared to the untreated sample).

Based on the assumption that 1 g of VS can be transformed in 0.5 L of biogas, the bioconversion efficiencies of the different treatments were calculated. The untreated digested CSTR effluent (Control) had a bioconversion efficiency of 36 %. The samples that were subjected to the AWO treatment (condition A, B and C) had a bioconversion efficiency of 61, 64 and 74 %.

In Figure 5, the cumulative biogas yield in function of time is presented. The arrows indicate the addition of 1 g VS.

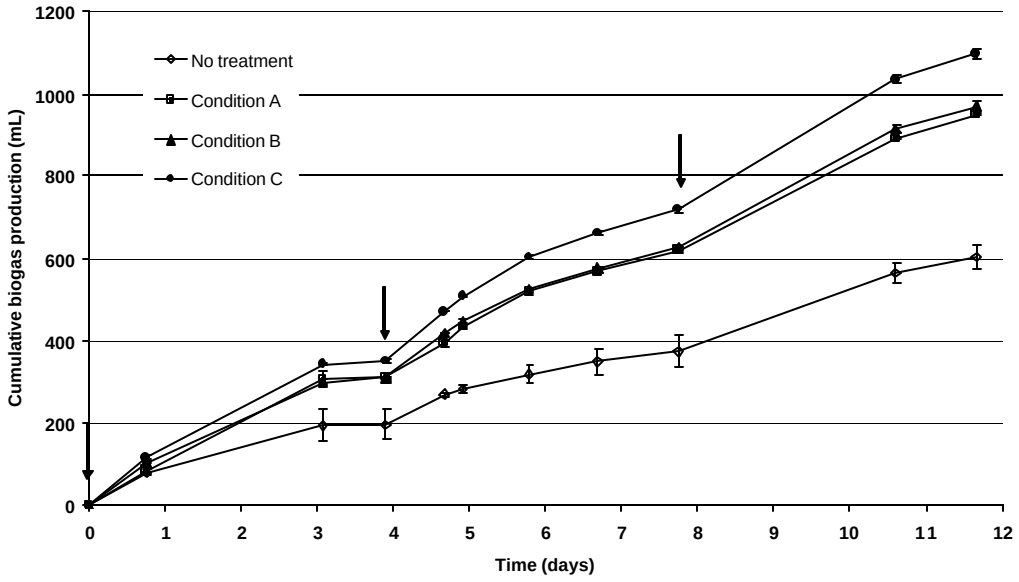

Figure 5: Cumulative biogas production (mL) in function of time. The digestions are indicated with arrows

At the end of the second digestion (t = 7.74 d), the supernatant of the batch fermentation tests was analysed on volatile solids content (VS). The biogas composition was determined and the percentage of methane and carbon dioxide were calculated. The results of these analyses are shown in Table 2.

Table 2: Effluent and biogas composition at the end of the second digestion (t = 7.74 d)

		Biogas composition	
	VS (g/L) supernatant	**CH_4 (%)**	**CO_2 (%)**
Control	1.15 ± 0.08	73 ± 6	27 ± 6
AWO condition A	1.46 ± 0.00	81 ± 1	19 ± 1
AWO condition B	1.66 ± 0.09	78 ± 3	22 ± 3
AWO condition C	1.79 ± 0.12	76 ± 1	24 ± 1

From Table 2 it can be seen that the percentage of methane in all the fermentation tests was between 73 and 81 %. This is relatively high and can be explained by CO_2 that is in solution due to overpressure in the reactor system. The VS concentrations in the supernatant of the different reactors after the second digestion are similar. This shows that despite the higher amount of solubilized VS in the wet oxidized materials (A-C), the biogas production from all substrates was readily without any inhibition. From this observation it can be concluded that the material in solution is largely (at least 70%) converted into biogas.

Previous results showed that the AWO-treatment is particularly interesting to convert lignin compounds into biodegradable carboxylic acids and further on into methane in a second digestion (Lissens et al., 2004). The lignin oxidation is generally higher (64-74%) at higher oxygen pressure and high alkalinity. This can be explained by the occurrence of high amounts of phenoxyl linkages in lignin, which are excellent radical mediators during oxidative processes (Dorrestijn et al, 2000). The main degradation products of lignin after wet oxidation have been reported to be carboxylic acids and partially CO_2 (Lissens et al., 2004a; Lissens et al., 2004b). Following soluble compounds were commonly found in the hydrolysates:

- Glucan (polymeric and monomeric glucose): 20-30% of the original cellulose
- Arabinoxylan (sum of arabinose and xylose): 60-80% of the original hemicellulose
- Lignin derivatives (mostly under form of carboxylic acids): 70% of original lignin
- Carboxylic acids (dominantly acetate): 4-7% of the total dry matter solids
- Fermentation inhibitors (furfural compounds): < 1% of the total dry matter solids

COMPARISON OF AWO-TREATMENT WITH H_2O_2-TREATMENT

The use of hydrogen peroxide and Fenton's reagent under ambient pressure and a temperature of 50°C to improve the biodegradability of the waste generated in the first compartment has been investigated previously by EPAS (TN 51.1, January 2000). In the study conducted by EPAS, the biodegradability as measured by total CO_2 and VFA production could be increased with only 11% (from 45% to 56%). Furthermore, a high amount of hydrogen peroxide was needed ($COD/H_2O_2 = 1$) which considerably impeded the applicability of Fenton reagent within the context of the project. Hence, it was concluded that the use of peroxide as a pre-treatment for the MELiSSA substrate is not recommended.

In this study, the AWO treatment involving the use of H_2O_2 under pressure (12 bars) and increased temperature (170°C) caused an increase of the biogas yield from the treated digested solids with 45-60% (320-360 mL for AWO condition B and C in the batch fermentation tests) compared to the untreated digested CSTR effluent (220 mL biogas). In this study, 0.25 g O_2/g DM waste was needed to create 12 bars oxygen pressure. This corresponds approximately to 10-12 mL H_2O_2 per liter of digested effluent.

ENERGY-COST CALCULATION OF THE AWO-TREATMENT

The major cost involved in the AWO process is the operational cost and more specifically the use of oxygen, either under the form of oxygen gas (or air) or under the form of liquid hydrogen peroxide. A cost-benefit analysis was recently made in case the AWO process would be applied to a full-scale anaerobic digestion (AD) plant (DRANCO, dry anaerobic composting) (Lissens et al., 2004). Based on a total oxygen requirement of 0.25 g O_2/g DM waste to create 12 bar oxygen pressure, the operational cost (including Na_2CO_3 costs and maintenance) for the AWO-AD process mounted to 9 €/ton waste and for the AD-AWO-AD

process to 3.7 €/ton waste (at 30% DM waste). For both processes, the capital costs for the AWO unit were about 2-3 €/ton waste. When the total costs made for the AWO process were compared with the gain in methane yield in a second digestion, it could be derived that the costs of the AWO process could be compensated by the energy gained from the extra methane recovery. When it is assumed that the composting costs after AD proportionally decrease with the gain in methane yield, an overall profit of 2.5-7 €/ton input waste was estimated. Furthermore, the calculation exercise did not take into account the potential oxygen and heat recovery (exothermic process) during AWO treatment.

Finally, as indicated already in TN 51.1 by EPAS, the H_2O_2 required for the AWO process could be electrochemically generated. Alternatively, pure oxygen or air could be extracted from the higher plant compartment of MELiSSA to thrive the AWO process.

Information on the WR^2 process for the destruction of BSE-contaminated waste for HACCP

In order to demonstrate that the proposed liquefaction system depicted in Figure 1 is 100% biosafe, it was decided during the latest progress meeting at DTU to evaluate by which extent the thermal technologies involved into the system (AWO technology and the liquefaction unit) comply with the new EU-regulation (No 1774/2002) for the destruction of BSE-contaminated waste. In case this can be demonstrated, it is assumed that biologically dangerous propagules of any nature can be completely destructed in the liquefaction compartment and thus 100% biosafety can be guaranteed.

Waste Reduction by Waste Reduction Inc. is headquartered in Indianapolis, Ind., where it develops and manufactures equipment that employs heat and alkali to totally eliminate path waste, tissues and carcasses (the WR2 process). The company operates an extensive research facility in Rensselaer (New York, United States). (http://www.wr2.net/process.htm).

The ***WR^2*** process was developed in 1992 by Drs. Gordon Kaye and Peter Weber, professors at the Albany Medical College in Albany, N.Y., and has been patented (US Patents 5,332,532, 6,437,211 B2, 6,472,580 B2, 6; European Patent # 0-677-205; other US and Foreign Patents

Pending). They originally invented the process as a way to properly dispose of biological tissue generated by biomedical and pharmaceutical research that contained small amounts of radionuclides and were classified as low-level radioactive waste.
Using a pressure vessel, Kaye and Weber perfected an alkaline hydrolysis process that converted the tissues into an aqueous solution containing their breakdown products, as well as the small amount of nuclide. The sterile, neutral, aqueous solution may be disposed of in a sanitary sewer in compliance with Nuclear Regulatory Commission regulations for radioactive waste or can be further processed by means of anaerobic digestion (and conversion into biogas).

The process reduces volume and weight by up to 98 percent, making it a superior alternative to incineration without air emissions or the potential for incomplete burns. It has been shown in lab tests to destroy Transmissible Spongiform Encephalopathy (TSE) agents, which cause diseases like Creutzfeldt-Jakob disease, "mad cow" disease, chronic wasting disease, and scrapie.

The ***WR²*** process uses alkaline hydrolysis at elevated temperature to convert the proteins, nucleic acids, and lipids of all cells and tissues, as well as infectious microorganisms, to a sterile aqueous solution of small peptides, amino acids, sugars, soaps, and electrolytes. The alkali itself is consumed in the process by generating the salts of the hydrolysis products. The only byproducts of the process are the mineral constituents (ash) of the bones.

The new EU regulation (No 1774/2002) recently approved two possible treatments to completely sanitize BSE-contaminated waste as an alternative to incineration or other disposal routes:

- Alkaline thermal hydrolysis: 150°C, 6 h, 4 bars pressure
- High pressure alkaline hydrolysis: 180°C, 40 min, 12 bars pressure

The WR company states that the resulting sterile solution after alkaline treatment is suitable for further processing by anaerobic digestion, thereby recovering the carbon under the form biogas and the application of the remaining liquid as fertilizer.

The AWO treatment as proposed above provide complete sanitation for pathogenic bacteria or viruses. Further research in collaboration with the DTU (Technical University of Denmark)

should indicate whether the AWO treatment also complies with the EU regulation for the complete sanitation of BSE-contaminated waste. Although the treatment times in the AWO treatment are considerably shorter (15 min versus 40 min in the WR^2 process), the AWO treatment also involves oxygen (12 bars) whereas the WR^2 process does not. Therefore, there are several indications that the AWO process can comply with the new EU regulation and can thus provide 100% biosafety in the system. Hence, the implementation of the AWO process in the liquefaction compartment could lower the residual waste stream to be treated in the high-pressure liquefaction unit and thus lower the energy demand of the system.

Selection of a chemical compound for the HACCP protocol

An electronic brain-storm meeting was held in the last week of March 2004 with all partners to decide which chemical would be most suitable as a test molecule for the HACCP protocol under development.

The most important proposals for a suitable test chemical were:

- tertiary alcohols (e.g. 2-methyl-2-butanol or MTBE)
- benzoic acid (aromatic compound, conservative)
- PCB's
- Polytetrafluoroethylene chippings (formation of dioxine and HF in subcritical reactor)
- Other plastics
- Antibiotics (beta-lactam)
- Hormones (estrogen, testosterone)

It was decided to go for a persistent, non-toxic chemical that is used on a common basis. Therefore, PCB's were not considered to be appropriate.

We think that two approaches can be put forward: either a chemical is chosen that occurs in food products or should preferentially be produced during the manufacturing of food products (e.g. benzoic acid), or alternatively, a chemical is chosen which can be potentially present in

the human faecal matter of the crew (e.g. hormones, antibiotics). A last approach could be a combination of both approaches (and thus the selection and testing of two chemicals).
The use of benzoic acid could be particularly interesting because this compound was found to be the most stable of the six aromatic carboxylic acids during hydrothermal treatment at 350 degrees C, showing negligible **degradation** after 1 h of hydrothermal treatment at 350 degrees C (Dunn et al., 2003). Thermal decomposition products of benzoic acid may also include toxic oxides of carbon and therefore this compound is of particular interest with regard to the hydrothermal liquefaction unit of Partner 4.

Finally, HACCP specialist Karel Verschueren was contacted via e-mail to assist in the choice of a chemical contaminant for the HACCP-protocol as agreed on the latest progress meeting at the DTU. He stated that recalcitrant compounds in nature are those that have a low water solubility and that have a high chlorination degree. One of those compounds is lindane (hexa chloro cyclohexane), which is furthermore highly toxic to aquatic organisms. The compound is a common pollutant in soils as a result of its use for the treatment of wood (in combination with penta chlorophenol). Further decisions on this matter need to be discussed on the next progress meeting (12th of May 2004 at ESTEC).

4 CONCLUSIONS AND FUTURE PERSPECTIVES

This work showed that the AWO process offers interesting features for implementation in a total converting and biosafe liquefaction compartment for MELiSSA. The process offers complete sanitation of the waste, is able to largely convert lignin into biodegradable carboxylic acids (Lissens et al., 2004) and can further increase the methane yield of the first methanogenesis unit with at least 50%. The AWO process is furthermore self-sustaining above a temperature of 160 degrees C (exothermic reaction) and the the majority (only 1 out of 12 parts oxygen added to the system are effectively consumed during AWO) of the oxygen added to the system can be recovered (as well as process heat).

Future work should be directed towards the construction of a solid HACCP protocol by considering the worst case scenario: biological contamination with BSE-contaminated waste and chemical contamination with one or two model test compounds (to be decided on the next progress meeting). For WP 1.6, the implementation of a solids retention system in methanogenesis unit 1 will be investigated to further increase the methane yield in the first unit.

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