EPAS n.v.

Eco Process Assistance

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**MELISSA**
**ESTEC/CONTRACT ECT/FG/CB/95.205**

**TN 26.1 & 26.2**

# Optimisation of the Melissa substrate biodegradation I:

## Optimisation of the analytical method, determination and quantification of the fermentation products

**January 1996**

**ir. Dries Demey**
**Ing. Peter Van Meenen**



Gedrukt op chloorvrij papier

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TECHNICAL NOTES 26.1 & 26.2
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## 1. INTRODUCTION

The results of the preliminary biodegradation test of human faeces by autochtonous bacteria were presented in TN22.5. A significant proteolysis of the proteins and a general hydrolysis of organic matter was noticed. Based on the results of these experiments it was decided to use the autochtonous bacteria present in the human faeces instead of co-cultures of the strains *Coprothermobacter proteolyticus*, *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*.

This report gives an overview of the different concepts and tests performed in order to improve the biodegradation of the human faeces by autochtonous bacteria.

**Table 1.1.** Schematic overview of the content of the technical notes 26.1 and 26.2.

**CONCEPT**

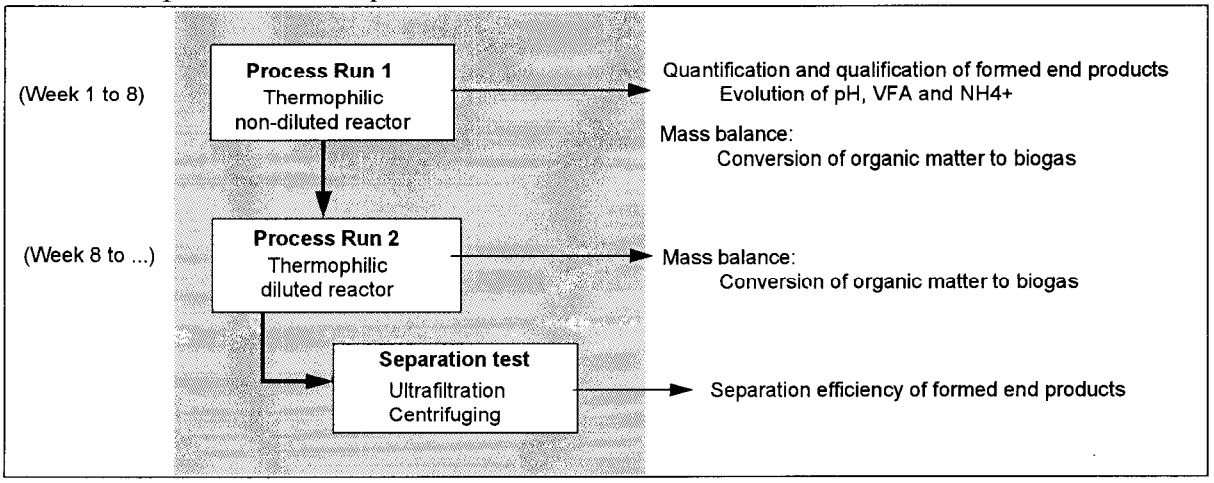
General considerations on anaerobic fermentation
- Anaerobic fermentation
- Anaerobic fermentation in view of the Melissa-loop

**EXPERIMENTS**

Analytical methods for the characterisation of the human faeces and the end products formed by anaerobic biodegradation of human faeces

Characterisation of the human faeces

Fed batch experiments and separation tests




Methanogenesis inhibition
- Qualitative tests to determine the process conditions for methanogenesis inhibition



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## 2. ANAEROBIC FERMENTATION

### 2.1. Process microbiology

In contrast to aerobic metabolism by which most organics are converted in a one-step process to final products such as $CO_2$ and $H_2O$, anaerobic digestion is a multi-step process in which substrates are subsequently degraded by certain groups of bacteria. Three groups of bacteria generally carry out the overall process which comprises four sub-processes.

Fermentative bacteria (or acidogens) are responsible for the first two subprocesses, i.e. hydrolysis and acidogenesis. Organic polymers are hydrolysed by exocellular enzymes into smaller units (monomers) which can enter the cells. In the cells, these monomers are further broken down through oxidation-reduction processes which result in the production of $CO_2$, $H_2$ and mainly volatile fatty acids such as propionic, butyric, iso-butyric, valeric and iso-valeric acid. To some extent formiate, lactate and alcohols such as butanol, ethanol and methanol may also be formed, depending on environmental conditions, e.g. substrates, pH, and $H_2$-partial pressure.

The second group are acetogenic bacteria or proton-reducing fermentors which break down the former products, i.e. fatty acids and alcohols into mainly acetate, $H_2$ and $CO_2$. It was demonstrated that the free energy change of the reactions is not favorable thermodynamically unless $H_2$ is efficiently removed by their counterparts (e.g. methanogens and sulfate reducing bacteria) and remains at sufficiently low concentrations. These microorganisms are therefore known as obligate $H_2$-producing or syntrophic acetogenic bacteria. For example, the degradation of propionate, butyrate and ethanol requires a $H_2$ partial pressure lower than $10^{-4}$, $10^{-3}$, and $10^{-1}$ atm respectively to make the Gibbs free energy exergonic. This leads to the concept of interspecies $H_2$ transfer, i.e. increase in $H_2$-flux between $H_2$-forming and $H_2$-consuming bacteria, which has proven to be more efficient when cells are in close proximity.
The third group are the methanogens which primarily cleave acetate or convert $H_2/CO_2$ into $CH_4$, $CO_2$ and $H_2O$. These bacteria are generally divided into two major subgroups : acetotrophic (or acetoclastic) and hydrogenotrophic bacteria. Approximately, one third and two thirds of the $CH_4$-production originates from the conversion of $H_2/CO_2$ and acetate, respectively.

### 2.2. Kinetic parameters and cell yields

The three main groups of bacteria involved in anaerobic digestion have very different growth constants, i.e. substrate conversion rate (q), half saturation concentration ($K_s$), maximum specific growth rate ($\mu_{max}$), cell decay rate (b) and cell yield (Y) as shown in Table 1. Based on these literature data, the following generalization can be made : the Y and $\mu_{max}$ of acidogens are about 5-10 times higher than those of acetogens and methanogens. The data imply that the balance of this anaerobic community will be quite delicate. Moreover, the cell yields of acetogens and methanogens could be a limiting factor for sludge growth, in absence of acidogens.

**Table 2.1.** Kinetic parameters (after Harper and Pohland (1986) and Pavlostathis and Giraldo-Gomez (1991) )

| Process | Substrate | q | $K_s$ | $\mu_{max}$ | b | Y |
|---|---|---|---|---|---|---|
| | | g COD/gVSS.d | mgCOD/l | $d^{-1}$ | $d^{-1}$ | gVSS/gCOD |
| **Acidogenesis** | Carbohydrates | 1.33-70.6 | 22.5-630 | 7.2-30 | 6.1 | 0.14-0.17 |
| **Acetogenesis** | $C_5$,$C_4$,$C_3$ fatty acids | 6.2-17.1 | 12-500 | 0.13-1.2 | 0.01-0.027 | 0.025-0.047 |
| **Methanogenesis** | Acetate | 2.6-11.6 | 11-421 | 0.08-0.7 | 0.004-0.037 | 0.01-0.054 |
| | $H_2/CO_2$ | 1.92-90 | $4.8*10^{-5}$-0.60 | 0.05-4.07 | 0.088 | 0.017-0.045 |

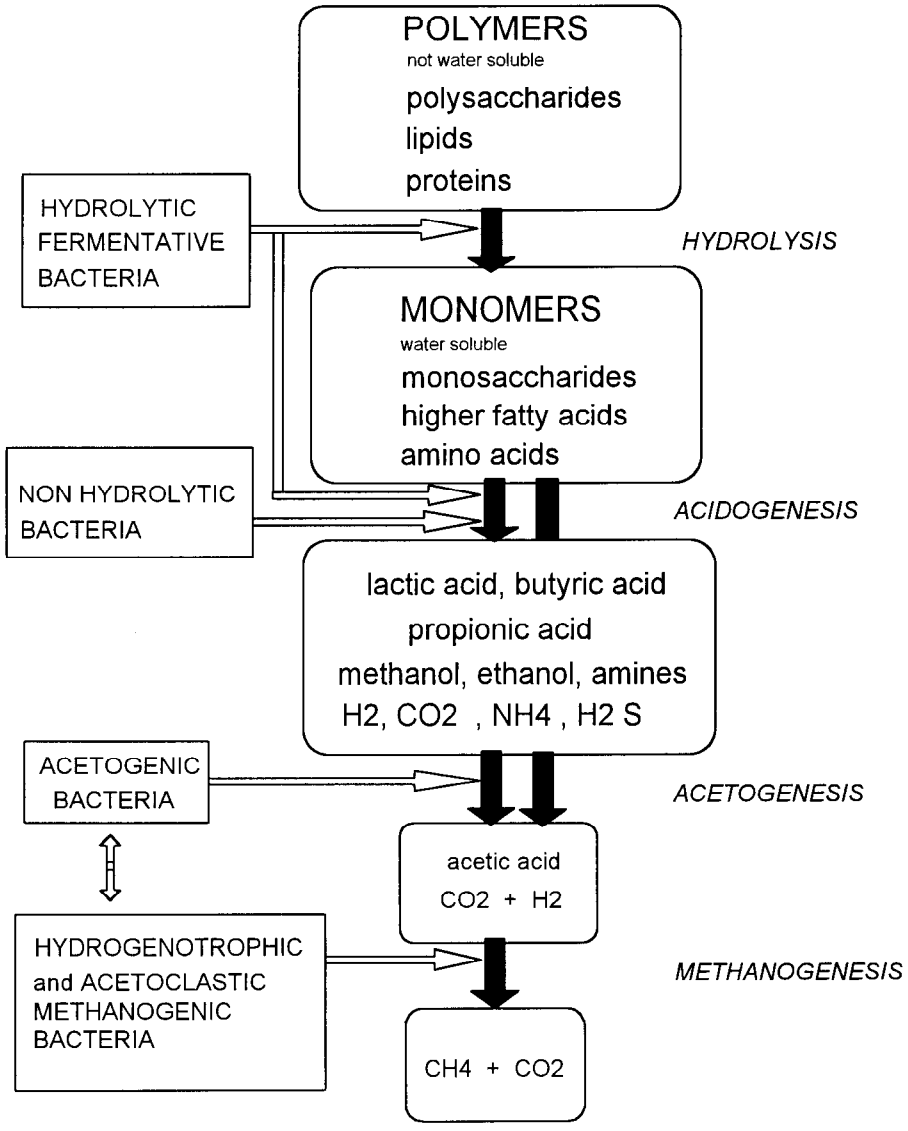



**Figure 2.1.** Anaerobic fermentation (after Verstraete (1995))

## 2.3. Environment related factors

### 2.3.1. Temperature

Anaerobic digestion can take place at mesophilic (25-40°C) or at thermophilic (45-60°C) temperatures. The advantages of anaerobic waste digestion at thermophilic temperatures compared to mesophilic temperatures are a higher digestion rate and a more efficient elimination of pathogens. A disadvantage of thermophilic digestion is the rather poor process stability. In some cases the effluent of anaerobic thermophillic digesters contains a relative high concentration of volatile fatty acids in the effluent, due to a lower conversion to methane. This is affected by sudden temperature increases, feed interruptions and shock loadings. When loading rates are high or retention times short, digestion at higher temperatures can be much more favourable compared to lower temperatures due to the higher growth rates of bacteria at higher temperatures.
A high temperature is stimulating the die-off of pathogenic organisms. Thermophilic systems remove pathogenic strains with a higher efficiency than mesophilic strains. Pathogen removal is the driving force behind the Danisch Biogas Programme to implement centralized thermophilic treatment for manure digestion, including industrial and household waste. Contact times of 1 hour at 55°C are sufficient to meet the prevailing sanitation standards in Denmark. (Bendixen, 1994)

### 2.3.2. Influence of the pH

In anaerobic digesters, methanogens appear to be most sensitive to pH. Methane production proceeds well as long as the pH is maintained between 6.6 and 7.6. Methanogenesis is often impaired at a pH below 6.5 and above 8.2, while most acidogens are still active at pH-values from 6.5 to 6.0.

### 2.3.3. Inhibitors

The pH can indirectly cause strong effects on methanogenesis since many compounds show an increased toxicity under the non-ionized form, e.g. volatile fatty acids, $NH_3$ and $H_2S$. Organic acids such as acetic, propionic and butyric acid often accumulate during digester failures.

The activity of each group of bacteria in the anaerobic association has a specific sensitivity for different inhibitors. Angelidaki et al. (1993) proposed the following inhibition factors for the different processes.

**Table 2.2.** Inhibition of the metabolic processes

| Process | Specific inhibitor |
|---|---|
| Enzymatic hydrolytic step | Inhibition by the sum of VFA |
| Acidogenic step | No inhibition |
| Acetogenic step | Inhibition by acetate and pH |
| Acetoclastic step | Inhibition by free ammonia and pH |

It is known that methanogenesis is strongly inhibited by high ammonium concentrations. Several threshold values are reported ranging from 2 to 7 g N/l. Free ammonia has been suggested as the active component causing inhibition. Consequently, the toxicity of ammonia is strongly dependent on pH and temperature. A level of 80 to 150 mg/l of free ammonia has been proposed as the minimum inhibitory level (Angelidaki et al. 1992).

One of the most negative chemicals in anaerobic digestion is sulfide. As the final product of the reduction of sulfate by a diverse group of sulfate-reducing bacteria, sulfide is not only very toxic to microbial associations, but eventually also causes problems for the utilisation of the produced biogas. Similar to ammonium, sulfide is toxic in the unionized form and therefore strongly dependent on pH and temperature. A concentration of 50 to 130 mg/l $H_2S$ is inhibiting 50 % of the methanogenesis (Kroiss and Plahl-Wabnegg, 1983, Oleskiewicz et al. , 1989).

## 2.4. Anaerobic fermentation in view of the Melissa-loop

Because it is of major importance for the MELISSA-loop to maximise the volatile fatty acid production and to inhibit the methane production, a special approach of anaerobic digestion is needed: instead of promoting the production of methane, the production has to be reduced. Several concepts to achieve this goal are summarised in Table 2.3.

**Table 2.3.** Process concepts to prevent methanogenesis

| **1. Consortium without methanogenic strains** | | |
|---|---|---|
| "Synthetic consortium" | Selection and combination of appropriate single strains. | Difficult to select suitable strains which can biodegrade human faeces.<br>What about competition between the strains? |
| "Cultivation" of a stable consortium without methanogenic bacteria | Based on the "natural selection" principle: methanogens present in the autochtonous strains will be repressed under stress conditions | What about re-inoculation with methanogenic bacteria present in the human faeces?<br>Autoclaving or pasteurisation of the faeces before feeding to the reactor? |
| **2. Consortium with methanogenic strains present but with an inhibited activity** | | |
| Activity inhibition | Operating the process under stress conditions for methanogens | |

A valuable concept to inhibit methanogenesis and to increase the volatile fatty acids concentration is schematically presented in Figure 2.2. A high ammonia concentration inhibits the conversion of volatile fatty acids tot methane. To prevent inhibition of the hydrolysis of the faecal material, the formed volatile fatty acids must be removed by a separation technique, such as ultrafiltration or centrifuging. Also ammonia will be removed. The ammonia needed to maintain a high ammonia concentration can be supplied by urea. The toxicity of urea to the global anaerobic process has to be examined.

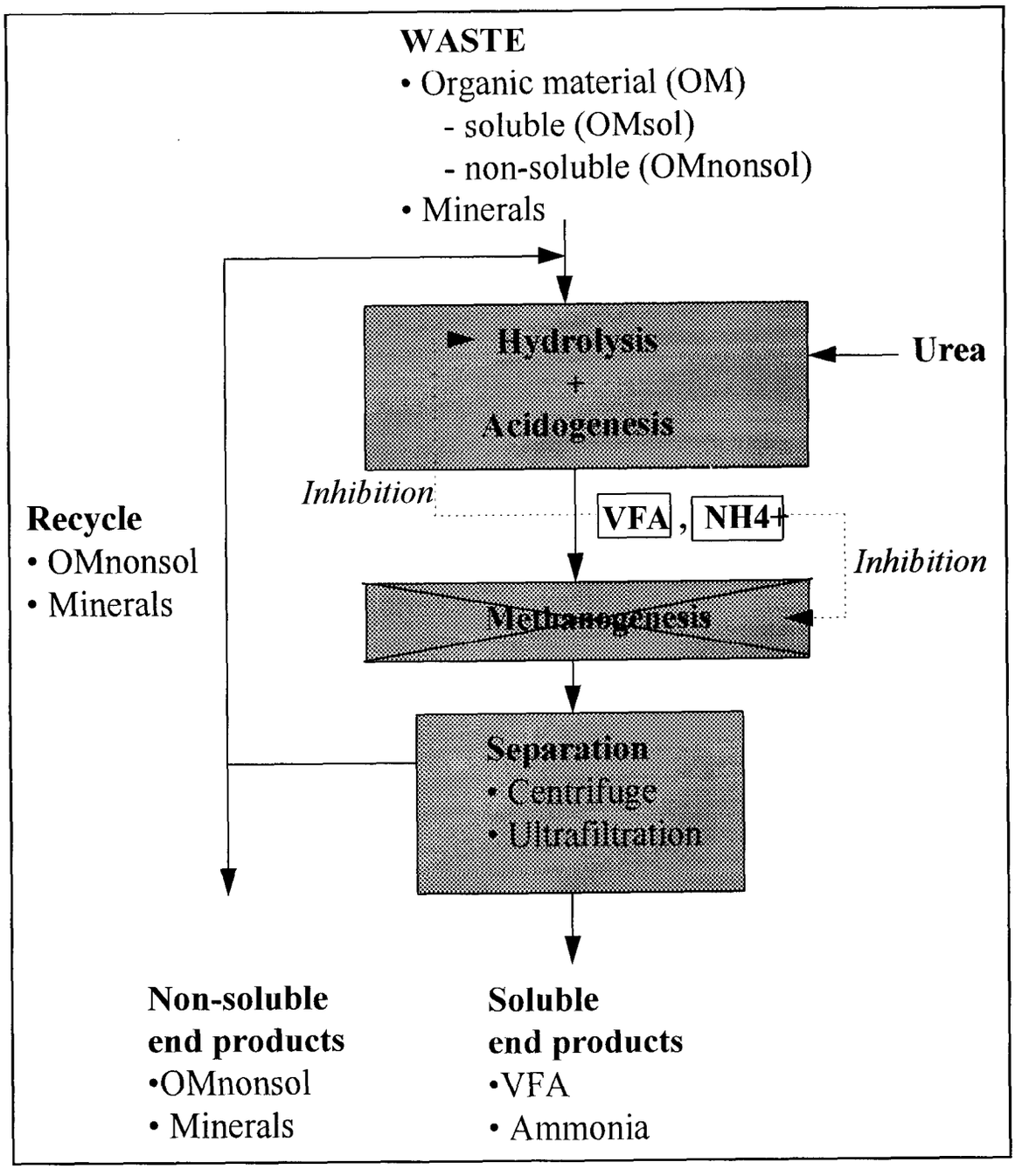



**Figure 2.2.** Methanogenesis inhibition

## 3. ANALYTICAL METHODS FOR THE CHARACTERISATION OF THE HUMAN FAECES AND THE END PRODUCTS FORMED BY ANAEROBIC BIODEGRADATION.

### 3.1. Routine measurement methods

The faeces and the end products were characterised by using established methods for the description of waste characteristics. Table 3.1 gives an overview of conventional parameters measured in anaerobic digestion research. These methods are quick and simple to perform and give valuable information about the biodegradability of organic materials. Monitoring and evaluation of biodegradation processes by means of these methods is well documented.

**Table 3.1.** Conventional parameters to describe waste characteristics and waste processing

| |
|---|
| Total COD |
| Soluble COD |
| Kjeldahl-nitrogen |
| Ammonia-nitrogen |
| Volatile fatty acids |
| Dry matter |
| Ash-content |

The *dry matter (DM)* of the sample was determined after 24 hours drying at 105° C. When the sample contains volatile fatty acids, the volatile fatty acids are evaporated during the drying period. This results in a dry matter content in which the amount af volatile fatty acids is not taken into accunt. In the report this dry matter content is called the *analyitical dry matter ($DM_A$)*. The sum of the amount of volatile fatty acids present in the sample and the analytical dry matter is called *total dry matter ($DM_T$)* is lower than the real dry matter content.

The *ash content* was determined after incineration at 450°C during a period of 3 hours.

*The $NH_4$+-N content* was determined by steam distillation in a Kjeltec 1002 apparatus under alkaline conditions. *The ($NO_3$- + $NO_2$-)-N content* was determined by steam distillation in a Kjeltec 1002 apparatus after reduction to $NH_3$ by the addition of Devarda alloy. *The Kjeldahl-N content* was determined similarly after complete destruction of the sample in strong acid.

The *chemical oxygen demand (COD)* corresponds to the amount of oxygen necessary for complete oxidation of all organic matter present in a given volume of sample. The organic content of the sample was subjected to oxidation by potassium dichromate in a strong acid medium (sulphuric acid plus silver sulphate) at a temperature of 150° C for two hours. The excess dichromate was then measured by back titration with ferrous ammonium sulphate. The *total COD ($COD_{tot}$)* was determined on the total sample, whereas *soluble COD ($COD_{sol}$)* was determined on a centrifuged sample.

*Volatile fatty acids (VFA)* were extracted with diethylether from acidified samples and determined by gas chromatography using a flame ionization detector coupled to a glass column containing chromosorb 101.

Methane and carbon dioxide were analyzed with an Intersmat IGC 120MB gas chromtograph connected to a Hewlett-Packard 3390A integrator. The GC was equipped with a dual column arrangement of Porapak (50-80 mesh) and a molecular sieve (60-80 mesh) and a thermal conductivity

detector. The column temperature was isothermic at 30°C, the carrier gas was argon, and the flowrate was 20 ml / min.

## 3.2. Determination of lipids and saccharides

Particularly for the MELISSA-project it is of interest to link the conventional parameters to the composition of the organic material in terms of proteins, lipids and poly- and monosaccharides content in order to simulate the MELISSA-loop.

Some suitable methods to determine those components in human faeces in a practically way were selected. The selection was based on a literature study. The main source of information were analytical methods for food characterisation.

### 3.2.1. Determination of the polysaccharides content

The polysaccharide content of the human faeces is of major importance because polysaccharides represent the main part of the difficultly biodegradable material. The group of polysaccharides can be divided into water soluble and water non-soluble components. In food chemistry, this type of polysaccharides are also indicated by the name "dietary fibres". The determination method proposed is based on the determination of water soluble, water non-soluble and total fibres in food.

Procedure A

One gram of sample is treated with heat-stable alpha-amylase (100°C, pH:6, 30 min), protease (60°C, pH:7.5, 30 min) and amyloglucosidase (60°C, pH:4.5, 30 min). The soluble fibre components are precipitated by adding 95 % ethanol. After filtration and washing with 78 % ethanol, 95 % ethanol and acetone, the dry residue corresponds to the total dietary fibre (TDF). Corrections for ash and protein are also carried out.

Procedure B

After enzymatic treatment, similar to procedure A, filtration and washing with distilled water, 95 % ethanol and acetone are carried out. The residue corresponds to the insoluble dietary fibre (IDF). Filtrate and washing liquid are saved and precipitated by adding 4 volumes of 95 % ethanol. The corresponding residue (soluble dietary fibre (SDF)) is washed with 78 % ethanol, 95 % ethanol and acetone. TDF is determined by the sum of SDF and IDF. Corrections for protein and ash on both residues are also performed.

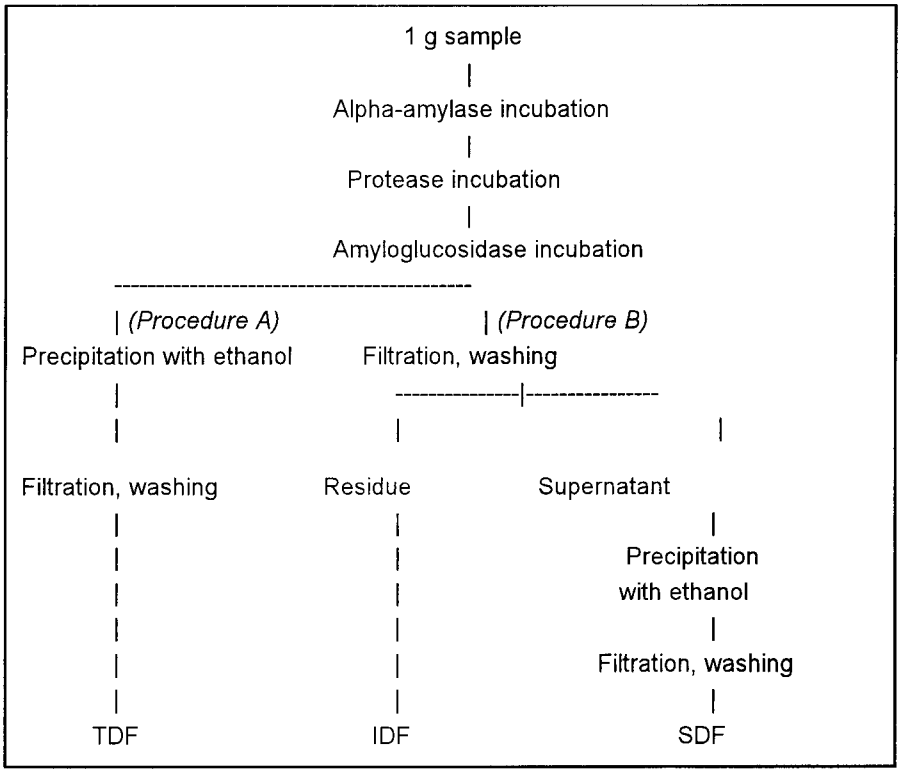



**Figure 3.1.** Analytic scheme for the enzymatic/gravimetric method to determine dietary fibre (Procedures A and B)

Although the fibre content of faeces is originating from undigested food, it was not possible to obtain accurate data that could be used in mass balance calculations. This will be further investigated.

### 3.2.2. Determination of lipids

The quantity of lipids present in a biological sample can be measured by the Soxhlet extraction method. The lipids present in the sample (5 g wet weight) are extracted with 100 ml of petroleumether (mixture of several ethers with a boiling point between 40°C to 60°C) in a Soxhlet apparatus. After extraction during a period of 24 hours, the solvent is evaporated and the remaining lipid fraction is determined gravimetrically.

Using this method for the quantification of lipids present in faecal material, accurate results were obtained.

## 4. CHARACTERISTICS OF THE HUMAN FAECES

Human faeces were used as a substrate to feed the reactor. The faeces were collected from volunteers with an age between 23 and 40 years. Immediately after collection the faeces were frozen and stored at minus 18°C. Table 4.1. shows the characteristics of the faeces used in the experiments.

**Table 4.1.** Composition of the human faeces

| | Unit | Average | Standard deviation | Standard Error | Number of measurements |
|---|---|---|---|---|---|
| **Dry Matter** | g/kg WW | 290 | 49 | 17 | 8 |
| **Organic Matter**[1] | g/kg DM | 861 | 13 | 4.3 | 8 |
| **Particular material**[2] | % of DM | 35 | 2 | 0.8 | 7 |
| **Soluble organic matter**[3] | % of DM | 28 | 5 | 2 | 7 |
| **$COD_{tot}$** | g/kg DM | 1270 | 172 | 65 | 7 |
| **$COD_{sol}$** | g/kg DM | 355 | 62 | 23 | 7 |
| **$NH_4^+$-N** | g/kg DM | 5 | 2 | 1 | 7 |
| **Organic nitrogen**[4] | g/kg DM | 46 | 4 | 1 | 7 |
| **pH** | | 7 | 0.1 | 0.04 | 7 |

(1) Calculation based on volatile solids determination
(2) Calculation based on suspended solids determination
(3) $COD_{sol}$ / $COD_{tot}$ - ratio
(4) Kjeldahl-N minus ammonia -N

An initial estimation of the molecular composition of the human faeces was calculated based on the molecular composition of proteins, lipids and carbohydrates and the occurrence of the components in the human faeces.

**Table 4.2.** Estimation of the molecular composition of human faeces

| | Formula | % (weight basis) |
|---|---|---|
| **Proteins** | $C_{16}H_{12}O_5N_4$ | 30 |
| **Lipids** | $C_8H_{16}O$ | 8 |
| **Carbohydrates*** | $CH_2O$ | 62 |
| **Human faeces** | $C_{39.3}H_{71.8}O_{25.6}N_{3.4}$ | |

*The concentration of carbohydrates was estimated by subtracting the protein content and the lipid content from the total organic matter.

The COD value of the human faeces can be calculated as follows:

$C_aH_bO_cN_d$

1 mole = 8 (4a + b - 2c - 3d) g COD
1 gram faeces (organic matter) = 1.34 g COD

Analytically a mean COD value of 1.48 g COD/g faeces (organic matter) was found. This value corresponds, taking the standard deviation into account, with the theoretically calculated value.

## 5. FED BATCH EXPERIMENTS

The aim of the fed batch experiments was to select in a natural way a stable consortium of bacteria, capable of hydrolysing the human faeces with a high efficiency.

The conditions in the reactor are different from the conditions in the human gastro-intestinal tract. When the human faeces are introduced into the reactor some bacteria species will be repressed by the strains which grow very well under the new conditions. After a period of time, a new population of bacteria will be established.

During the experiments the formation of end products was quantified and determined. The conversion efficiency of organic matter to biogas was calculated.

### 5.1. Materials and methods

#### 5.1.1 Reactor configuration

A completely mixed and heated reactor with a total volume of 1.5 litre was used for the experiments. A gas measurement system and sampling system were provided. The configuration of the set-up is schematically presented in Figure 5.1.

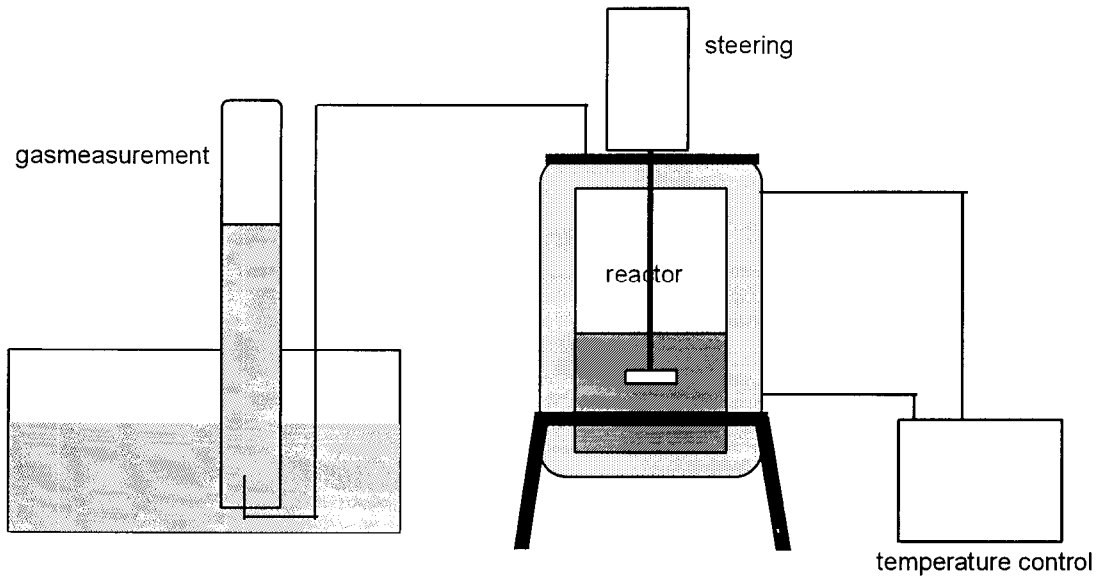



**Figure 5.1.** Reactor configuration

### 5.1.2. Process conditions

Two different process runs were performed upto this moment.
During the first process run the faeces fed to the reactor were not diluted. The analytical dry matter content ($DM_A$) of the reactor was equal to 12.9 ± 0.9 %. The total dry matter ($DM_T$) content including the volatile fatty acids was equal to 17.3 ± 1.1 %.
At the start of the second process run the reactor was diluted two times because of the fact that the non-diluted reactor content could not be centrifuged to separate the soluble end products from the non-biodegraded materials. The faeces fed to the reactor were diluted five times.

*Process run 1:*

The reactor was operated under thermophilic (55°) conditions. This temperature was chosen in order to reduce pathogenic organisms. At the start of the first process run, the reactor was filled with 105 grams organic matter of faecal origin. Only then a amount of 150 ml water was added to prevent the drying out of the faeces. The reactor was intermittently fed with a total amount of 50 grams of organic matter. After two weeks the reactor was operated at a regime according to the process parameters indicated in Table 5.1. The reactor content and the feed were not diluted. The reactor was operated during 57 days at this regime. The reactor was fed every two days.

**Table 5.1.** Process parameters

| **Parameter** | | **Value** |
|---|---|---|
| **Volumetric Load** | g HF/day.liter HF | 22.12 |
| | g OM/day.liter HF | 5.72 |
| **Mean Residence Time** | Days | 25 |
| **Temperature** | °C | 55 |
| **Initial pH** | | 7 |

*Process run 2:*

Because of the fact that the non-diluted reactor content could not be centrifuged to separate the soluble end products from the non-biodegraded materials, the reactor content was diluted 2 times. At the start of the second process run, the kinetics of the conversion of organic matter to biogas were determined.

## 5.2. Results

### 5.2.1. Evolution of the measured parameters during Process Run 1

The evolution of the different measured parameters is presented in Figures 5.2 to 5.6.

The mean concentration of ammonia in the reactor content was equal to 6.5 ± 0.6 g/l and was very stable during the process as shown in Figure 5.3.

The concentration of volatile fatty acids was high (41.2 ± 6.2 g/l) as illustrated in Figure 5.4. Despite the high volatile fatty acid concentration, the pH was very stable (Figure 5.5). Only during the 14 last days of the process a pH drop of 0.4 units was noticed. This means that the buffer capacity of the

reactor content was high. At day 18 a drop into volatile fatty acids concentration was measured. This decrease was possibly due to analytical problems on VFA measurement.

During the first 24 days of the process about 40 % of the total nitrogen was organic nitrogen (Figure 5.6). At the end of the process only 33 % of the nitrogen was organic nitrogen. When this value is compared to the organic nitrogen in human faeces (91 % of the total nitrogen), it can be concluded that proteolysis increased.

About 50 % of the organic matter present in the reactor was soluble organic matter (Figure 5.6). Human faecal material contains 25 % of soluble organic matter.

The feeding and sampling regime of the reactor was stable. There was no significant increase or decrease in dry matter ($DM_A$) and organic matter during the experiment.

During the start-up of the first process run, methane was present in the produced biogas (approx. 75% $CH_4$). After the start-up period no methane was produced during the process run. This indicates that there was a die-off of methanogenic bacteria or an inhibition of the activity of the methanogenic bacteria. To justify this hypothesis closed bottle tests were set up (see point 7).

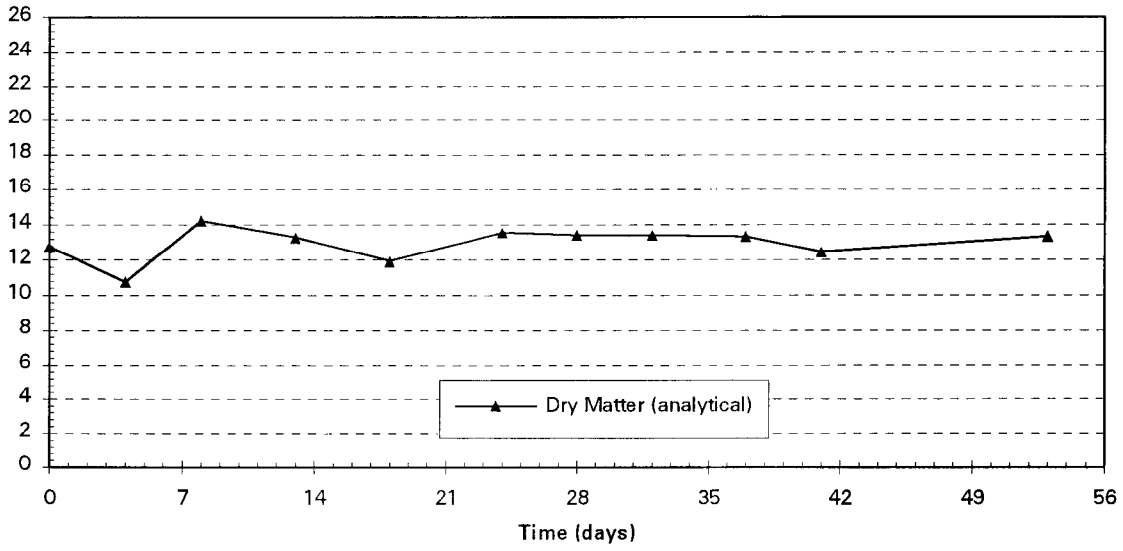



**Figure 5.2.** Evolution of the dry matter (% of wet weight) and the ash content (% of dry matter)

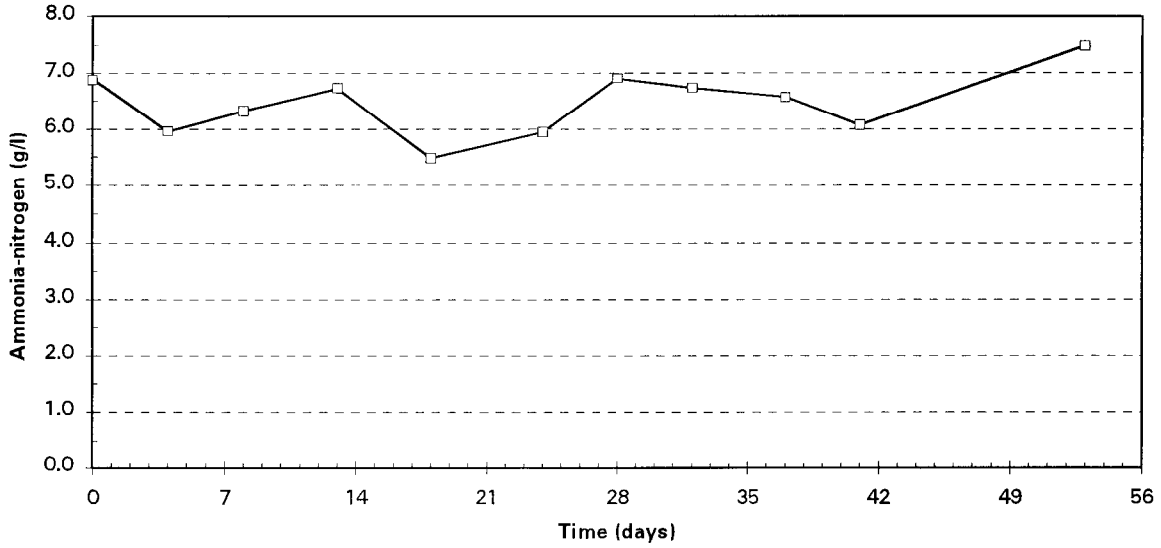



**Figure 5.3.** Evolution of the ammonia concentration (g/l)

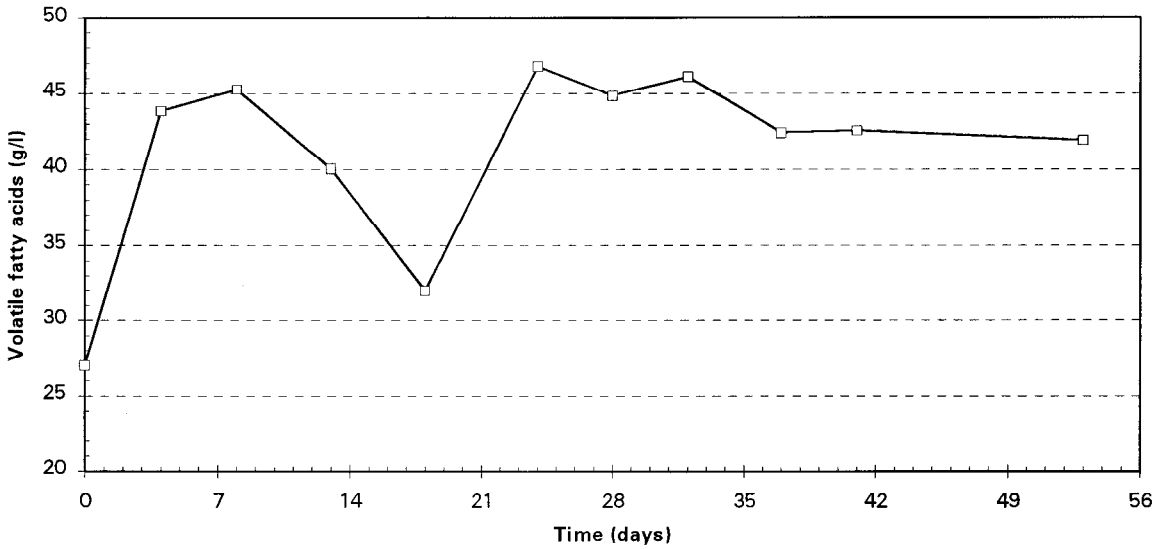



**Figure 5.4.** Evolution of the volatile fatty acids concentration (g/l)

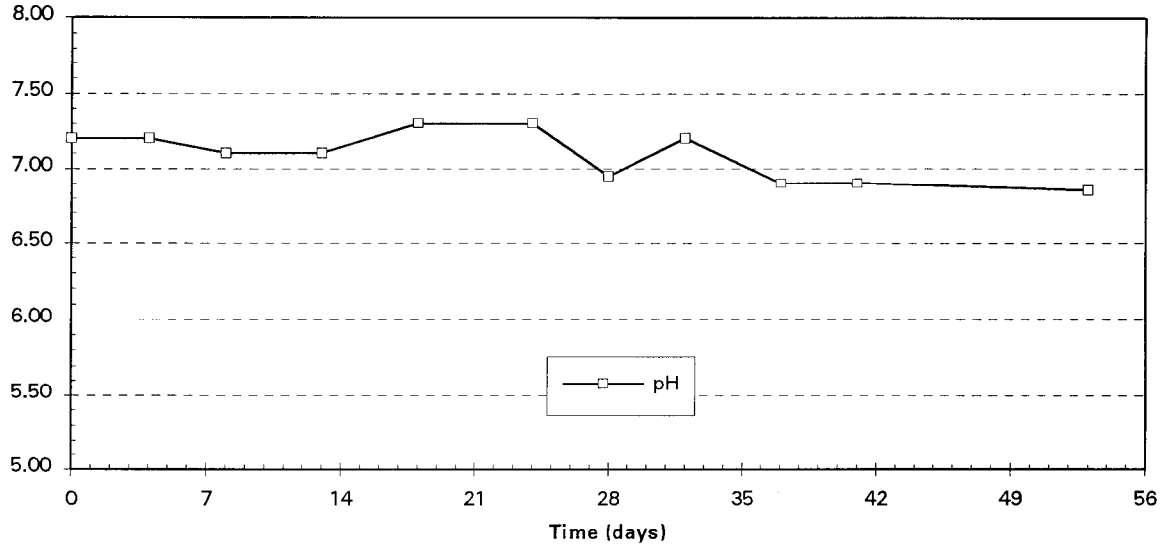



**Figure 5.5.** Evolution of the pH

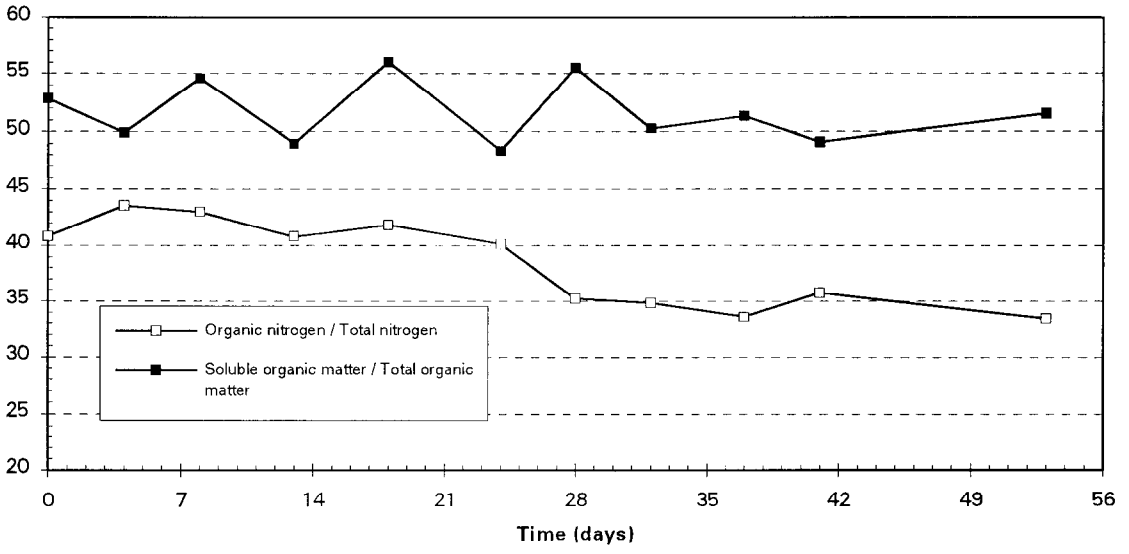



**Figure 5.6.** Evolution of the efficiency of the global hydrolysis and the proteolysis

### 5.2.2. Mass balances for the conversion of organic matter into biogas (Process Run 1)

A mass balance, based on the gas production during Process Run 1, was calculated. The basic parameters for the calculation are summarized in Table 5.2. The results are given in Table 5.3.

**Table 5.2.** Basic parameters for the mass balance calculation

| Period | 57 days |
|---|---|
| Biogas | |
| Total production | 2385 ml |
| Composition | 100 % CO2 |
| Total feed | 80 g organic matter |
| Amount of organic matter converted to biogas (1) | 2.69 gram |
| Percentage of organic matter converted to biogas | 3.36 % |

(1) : Using the Ideal Gas Law (formula : P.V = n.R.T), the amount of carbon in the biogas originating from the biodegradation of organic matter can be calculated:

22.4 litre CO2 ~ 1 mole of CO2 ~ 44 g of CO2 ~ 12 g C

2385 ml of CO2-gas contains 1,27 g C or 2.69 g organic matter

It is assumed that 1 gram organic matter contains 47,2 % (w/w) carbon.

**Table 5.3.** Mass balance

| | **Dry matter** | Ash | **Volatile solids** |
|---|---|---|---|
| Initial | 100 | 13.9<br>(13.9% of DM) | 86.1 |
| Conversion | | | into biogas : 86.1 x 0.0336 = 2.90 g<br>Organic nitrogen to $NH_4^+$-N : 4.3 g |
| End | 97.10 | 13.9 ash + 4.3 $NH_4^+$-N = 18.2<br>(18.74 % of DM) | 78.90 g |

### 5.2.3. Conversion of organic matter into biogas (Process Run 2)

Based on gas production measurements, the kinetics of the conversion of organic matter to biogas was calculated. The theoretical biogas production can be calculated, using the formula proposed by Buswell :

$$C_nH_aO_bN_C + \left(\frac{4n-a-2b+3c}{4}\right) H_2O \rightarrow \left(\frac{4n+a-2b-3c}{8}\right) CH_4 + \left(\frac{4n-a+2b+3c}{8}\right) CO_2 + c\ NH_3$$

with : biogas production: 1075 ml per gram organic matter
composition: CH4 (43.6 %) and CO2 (56.4 %).

The biogas produced during the experiments contained 38 % CH4 and 62 % CO2.

Figure 5.7 shows the evolution of the conversion of organic matter to biogas during 7 days. The curve was fitted to a two fractorial decay model:

$$N_t = N_s.e^{-ks.t} + N_f.e^{-kf.t}$$

Results are given in Table 5.4.

**Table 5.4.** Two fractorial decay model

| | Slow fraction | | Fast fraction | |
|---|---|---|---|---|
| | **Value** | **Error** | **Value** | **Error** |
| **Fraction of total mass (g/g OM)** | $N_s = 974.2$ | 0.27 % | $N_f = 26.7$ | 9.74 % |
| **Conversion constant ($d^{-1}$)** | $k_s = 0.0065$ | 8.13 % | $k_f = 2.12$ | 26.70 % |

Only 2.6 % of the organic matter was rapidly converted into biogas, the rest is converted slowly to biogas. Using this model, a prediction of the organic matter conversion into biogas was made. It seems that theoretically a period of about hundred days is needed to convert 50 % of the organic matter into biogas (Figure 5.8).

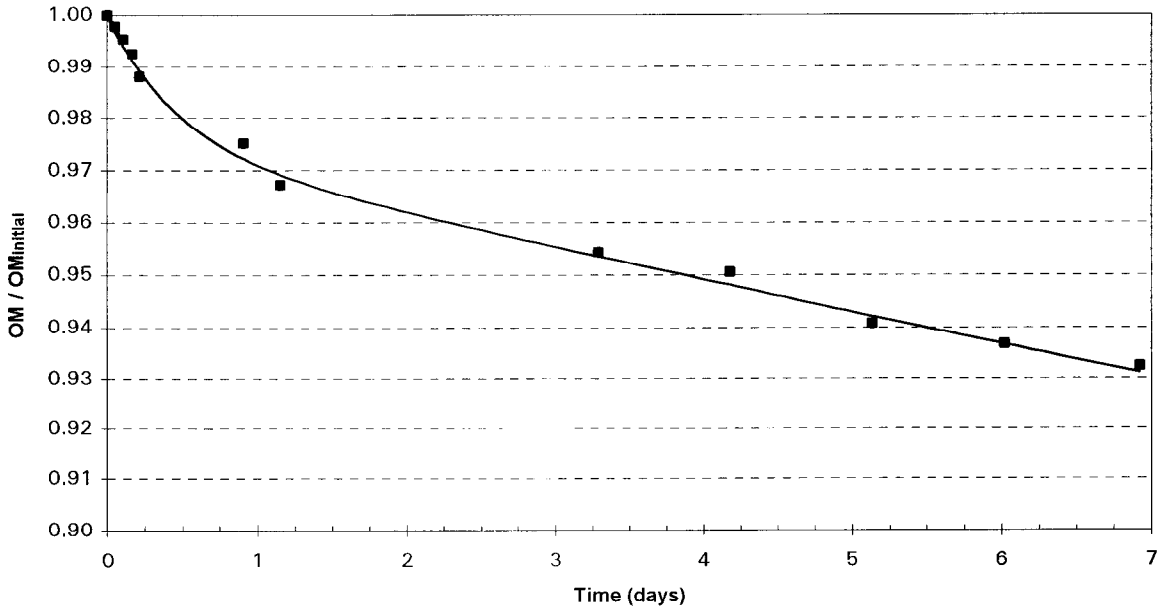



**Figure 5.7.** Evolution of the conversion of the organic matter to biogas (measured data and fitted curve).

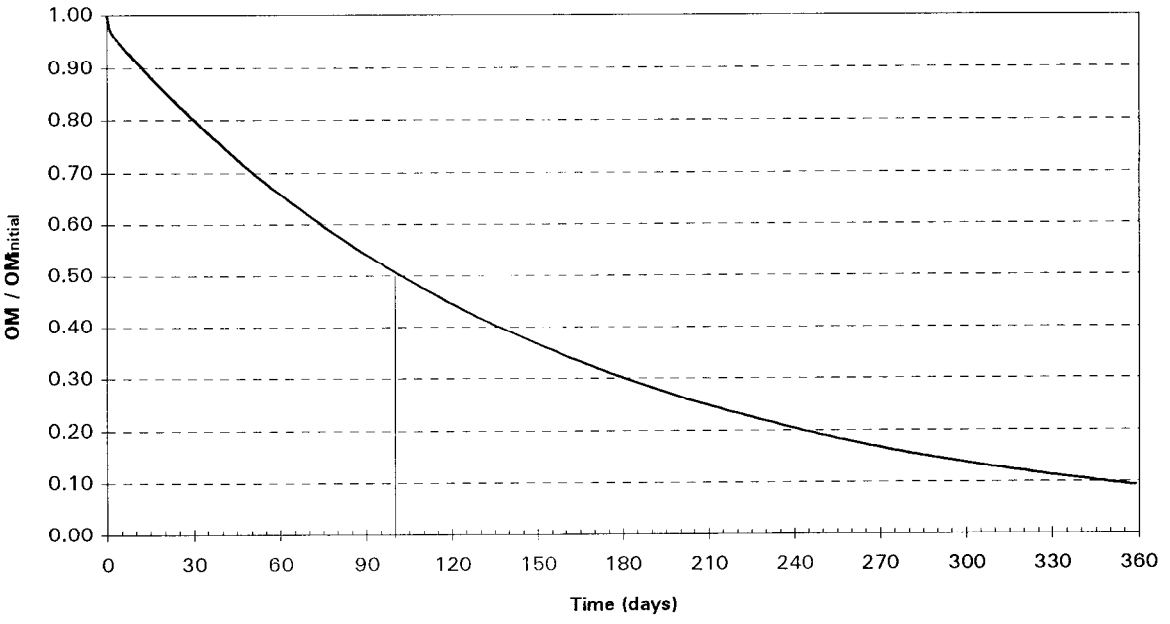



**Figure 5.8.** Theoretical prediction of the conversion of organic matter to biogas.

## 6. SEPARATION TESTS

### 6.1. Materials and methods

Separation of the soluble hydrolysis and fermentation products is necessary, because in the second compartment of the Melissa-loop, volatile fatty acids and ammonia are used to produce biomass. Preliminary separation tests, using a high velocity centrifuge and an ultrafiltration pressure cell, were carried out.

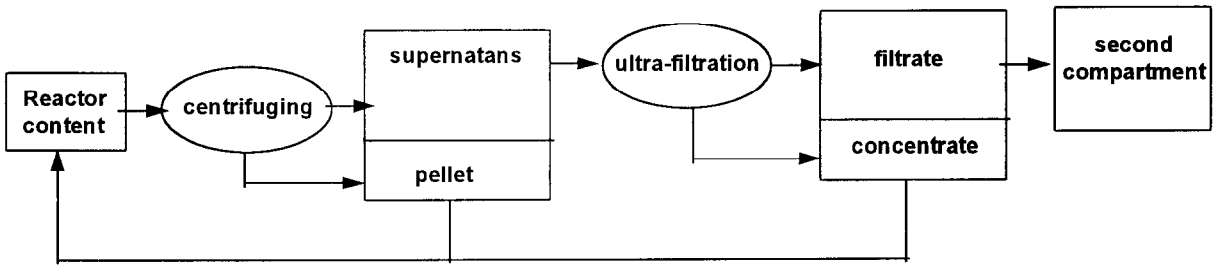



**Figure 6.1.** Separation scheme

#### 6.1.1. Centrifuge

A sample (100 ml) of the reactor content was centrifuged with a high speed centrifuge at an angular velocity of $12.10^3$ rounds per minute. Samples with a dry matter content of 11 % and 6.9 % were centrifuged during 15 minutes. The efficiency of the removal of soluble organic matter was determined.

#### 6.1.2. Ultrafiltration cell

A schematic presentation of the pressure cell is given in Figure 6.1. The cell permits to test filtration efficiency of several membrane types at a pressure of maximum 5 bar. The pressure used in the tests was equal to 3 bar.

An ultrafiltration membrane with a cut-off of 8000 Dalton was tested. Because of the poor results, a Whatman filter with a pore diameter of 8 µm was used in a second experiment.

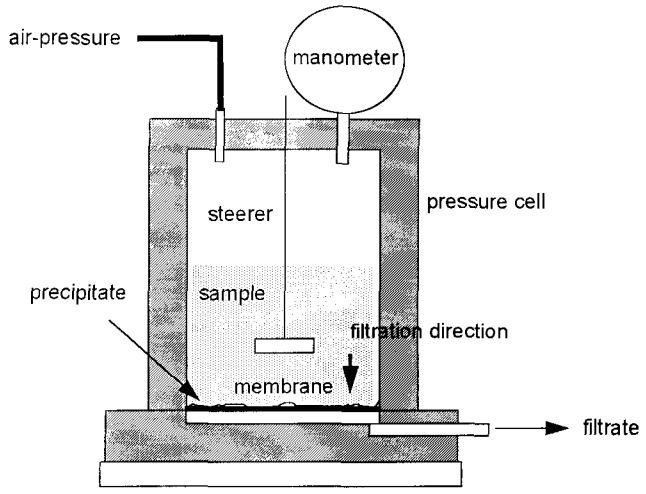



**Figure 6.2.** Schematic presentation of the ultrafiltration pressure cell.

## 6.2. Results

### 6.2.1. Centrifuge

The results of the experiments are presented in Table 6.1. When the analytical dry matter content of the sample was equal to 12.9 %, the volume of the supernatant was 54 % of the initial sample volume. When the sample had a analytical dry matter content of 5.5 %, the supernatant volume was 68 % of the initial sample volume. Ammonia was separated with an efficiency of 62 % and 46 % when the initial dry matter of the sample was equal to 5.5 % and 12.9 %, respectively. Approximately 24 % of the organic nitrogen was retained in the supernatant volume for the sample with the lowest dry matter content, and 45 % for the sample with the highest dry matter content. It was not yet possible to retain the non-biodegraded fraction of nitrogen in the precipitate.

**Table 6.1.** Separation efficiency of the soluble end products by centrifuging the reactor content (two different dry matter concentrations)

| Parameter | Unit | Diluted 5.5 % $DM_A$ | | Non-diluted 12.9 % $DM_A$ | |
|---|---|---|---|---|---|
| | | Concentration in supernatant | Percentage removal | Concentration in supernatant | Percentage removal |
| Volume supernatant | % of total | 68 | - | 54 | - |
| Volume precipitate | % of total | 32 | - | 46 | - |
| Dry matter | % | 1.5 | 21 | 2.0 | 9 |
| NH4+-N | mg/l | 1965 | 62 | 2020 | 46 |
| Organic nitrogen | mg/l | 559 | 24 | 903 | 45 |
| Volatile fatty acids | mg/l | 13445 | 74 | 10423 | 48 |
| Acetic acid | mg/l | 6116 | 76 | 4293 | 70 |
| Propionic acid | mg/l | 2330 | 74 | 950 | 50 |
| Iso-butyric acid | mg/l | 820 | 74 | 630 | 39 |
| Butyric acid | mg/l | 2485 | 73 | 2789 | 40 |
| Iso-valeric acid | mg/l | 1563 | 73 | 1572 | 35 |
| Valeric acid | mg/l | 131 | 71 | 189 | 34 |

### 6.2.2. Ultrafiltration

Ultrafiltration, using the membrane with a cut-off capacity of 8000 Dalton, was not successful. No permeate passed the membrane. In case the Whatman filter was used, 80 % of the initial volume passed the filter. Table 6.2 shows the removal efficiency of the organic matter and the nitrogen. The soluble organic matter passed the filter for 100 %, but also 40 % of the non-soluble material. Approximately 80 % of the initial ammonia and the organic nitrogen was found in the permeate.

**Table 6.2.** Removal efficiency by ultrafiltration of the end products present in the supernatant

| Parameter | | Removal efficiency (%) |
|---|---|---|
| NH4+-N | mg/l | 82 |
| Organic nitrogen | mg/l | 80 |
| Volatile fatty acids | mg/l | 100 |

### 6.2.3. Conclusions

The separation test demonstrates the positive effect of the dilution of the reactor content on the separation efficiency of ammonia and volatile fatty acids. The separation efficiency of the centrifuging step for ammonia was 62 % and for volatile fatty acids 74 %. During the ultra-filtration step, ammonia and the organic nitrogen passed for 80% the 8 µm membrane and the volatile fatty acids for 100%. It wasn't possible to separate the ammonia nitrogen and the volatile fatty acids without remaining fractions of organic nitrogen.

## 7. INHIBITION OF METHANOGENESIS

During the fed-batch experiment "Process run 1" no methane was produced. This can be explained by the suboptimal growth conditions in the reactor for methane producing bacteria, resulting in an inhibition of the activity of the methane producing bacteria or even a die-off. However, from the beginning of Process run 2 the reactor content was diluted 2 times to 6 % dry matter and methanogenesis restarted. This indicates that reactivation of the methanogenic activity is possible by supplying new feed or changing process conditions such as a lower ammonia and/or volatile fatty acids concentration. In order to check this hypothesis a closed bottle test was performed.

### 7.1. Experimental set-up of the closed bottle test

Dilution series of the different tested media (see Table 7.1) with a dilution factor from 1 to 10 were made. Flasks of 100 ml content were filled with 60 ml of the diluted medium.

The dry matter of the reactor content (RC) was equal to 6 %. The undiluted ammonia concentration was 1.5 g/l and the volatile fatty acid concentration was 8.2 g/l.

**Table 7.1.** Schematic overview of media tested in the methanogenesis inhibition test

| | |
|---|---|
| RC | Reactor content (60 ml) |
| RC+MI | Reactor content (60 ml) + methanogenic inoculum (10 ml) |
| MM+MI | Mineral medium (50 ml) + VFA (8.2 g/l) + Ammonia (1.5 g/l) + methanogenic inoculum (10 ml) |

## 7.2. Results

Figure 7.1 shows that already in the non-diluted reactor content (RC) and reactor content with the methanogenic inoculum (RC+MI) methanogenesis occurred. This means that methanogenic bacteria were still present in the reactor after the first process run. Probably, their activity was inhibited by the high ammonia concentration. Further experiments to confirm this hypothesis are in progress.

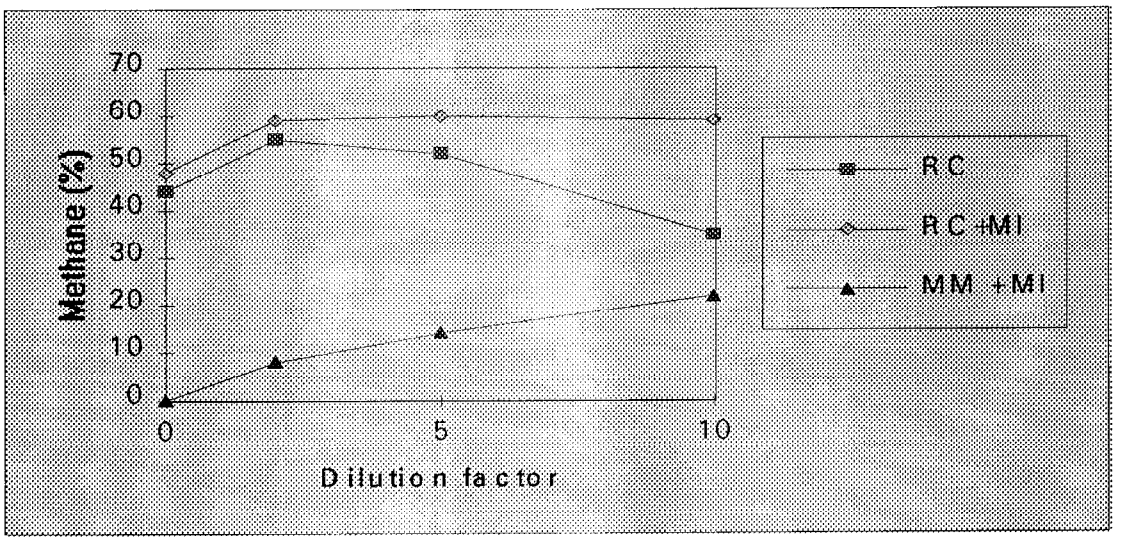



**Figure 7.1.** Evolution of the methane concentration in function of the dilution factor.

## 8. CONCLUSIONS

Based on the results retrieved from the first process run (undiluted reactor) a mass balance was calculated. it was assumed that one third of the faecal material consists of bacterial cells and that the amount of input of the reactor equals the output. The protein content was estimated by multiplying the organic nitrogen content with a factor 6.25. The amount of carbohydrates and lipids was estimated by subtracting the amount of proteins and volatile fatty acids from the total organic matter content. The conversion of organic matter into biogas was equal to 3.36 % (see Table 5.2).

Figure 8.1 gives a schematic presentation of the mass balance. In Table 8.1 the breakdown efficiencies of proteins and lipids + carbohydrates obtained during the first process run are compared with the results obtained in previous closed bottle tests (TN22.5). The breakdown efficiencies of the fraction of the non-bacterial organic matter obtained during the fed batch tests are comparable with the results obtained during the closed bottle tests. The mass balances for the closed bottle tests were calculated in a different way than the actual mass balance because the closed bottle tests permitted no accurate calculation of the conversion of organic matter into biogas.

**Table 8.1.** Breakdown efficiencies (%) of total and non-bacterial proteins and carbohydrates + lipids retrieved during the first process run with the undiluted reactor content (17.3 % ± 1.1 % $DM_T$)

| | Process Run 1 Undiluted reactor | Closed bottle tests TN22.5 |
|---|---|---|
| **Total proteins** | 59 | 47 |
| **Total carbohydrates and lipids** | 40 | 30 |
| **Non-bacterial proteins** | 89 | 89 |
| **Non-bacterial carbohydrates and lipids** | 61 | 57 |

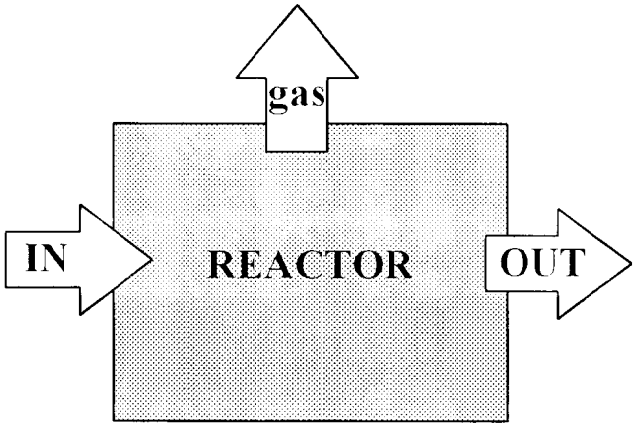
The biogas production, mainly carbondioxide, was very low and favourable for the Melissa - concept (see Table 5.2). An efficient degradation of proteins by autochtonous bacteria under thermophillic conditions was noticed, but the biodegradation efficiency of lipids and carbohydrates needs to be improved. Therefore, a thermophilic reactor will be inoculated with an inoculum obtained from a anaerobic thermophilic solid waste reactor. The reactor is fed with household refuse containing a lot of fibrous material (vegetables, fruit, paper). The inoculum contains cellulolytic strains, but also methanogenic strains. The possibility that methane production will occur after inoculation is high. In order to prevent this, the reactor will be operated under "stress"-conditions as illustrated in Figure 2.2. The experiments showed also that the faecal material has to be diluted to have an optimal separation of the end products by centrifuging and ultrafiltration.

Besides the optimalisation of the biodegradation process, some additional experiments are in preparation:

- Mesophilic reactor with autochtonous strains;
- Effect of adding cellulose to the reactors;
- Optimalisation of the separation of the end products;
- Enumeration of the amount of biomass in the reactors;
- Screening for pathogenic strains present in the effluent.

biogas production : 29 g organic matter converted

| | Component | gram | % |
|---|---|---|---|
| *OMi* | Organic matter | 861 | |
| *Ashi* | Ash | 139 | |
| *TNi* | Total nitrogen | 51 | |
| *ONi* | Organic nitrogen | 46 | (90) |
| *ANi* | Ammonia-nitrogen | 5 | (10) |
| *VFAi* | Volatile fatty acids | 28 | |
| *Pi* | Total proteins | 288 | |
| *Pti* | Bacterial | 96 | (1/3) |
| *Pbi* | Non bacterial | 192 | (2/3) |
| *LCi* | Lipids + Carbohydrates | 545 | |
| *LCti* | Bacterial | 182 | (1/3) |
| *LCbi* | Non bacterial | 363 | (2/3) |




| | Component | gram | % |
|---|---|---|---|
| *OMo* | Organic matter | 832 | |
| *Asho* | Ash | (139 + a.$NH_4$-salts) | |
| *TNo* | Total nitrogen | 51 | |
| *ONo* | Organic nitrogen | 18.9 | 37 |
| *ANo* | Ammonia-nitrogen | 32.1 | 63 |
| *VFAo* | Volatile fatty acids | 319 | |
| *Po* | Total proteins | 118 | |
| *Pto* | Bacterial | 96 | 81 |
| *Pbo* | Non bacterial | 22 | 19 |
| *LCo* | Lipids + Carbohydrates | 325 | |
| *LCto* | Bacterial | 182 | 56 |
| *LCbo* | Non bacterial | 143 | 44 |

**Figure 8.1.** Mass balance

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