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MELiSSA

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TECHNICAL NOTE 39.3

Liquefying Compartment
- Analysis, Stoichiometric and modelling approach -

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T.N. 39.3: Liquefying compartment

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Introduction

The liquefying compartment is probably the most important compartment of the MELiSSA loop. Because it is the first compartment of the loop and because it produces quite all the substrates used by the further compartments, its efficiency drives the efficiency of the overall loop [TN 17.2].
In the modelling approach of the complete loop, the liquefying compartment was described by theoretical black-box stoichiometries, and was assumed to have an ideal efficiency of 100% of faeces degradation [TN 32.3; Poughon et al., 1994; Poughon et al., 1997]. This description was in fact far from the current reality.

The purpose of this technical note is first to review the current state of art of the studies for the compartment and then to establish from this knowledge and from experimental results obtained by EPAS, at least, the basis for the building of the mass-balanced representation and of a dynamic model of the liquefying compartment.

I - MELiSSA liquefying compartment - current state of art.

I.1 - The selected strains

In their first description (Mergeay, 1988), the MELiSSA compartments were defined as axenic cultures of micro-organisms. The strains selected for the liquefying compartment were thermophilic (60°C) cellulolytic strains namely *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*. Thermophyllic strains were chosen in order to avoid the development of autogenous strains and pathogens.

Cl. thermocellum easily converts cellulose and hemicellulose into ethanol, acetic acid, CO_2 and H_2 [TN 7.1]
Cl. thermosaccharolyticum degrades dextrins, pectins and starch to the following end products: acetic acid, butyric acid, lactic acid, H_2 and succinic acid [TN 7.1].

Because of the low proteic activities of these two strains, a thermophillic and proteolytic strain were searched and isolated [TN 15.1, TN 15.3] from a Dry Anaerobic Composting Reactor (DRANCO). The strain selected was taxonomically studied and identified as *Copronothermobacter proteolyticus I8*. It grows too on carbohydrates and produces acetic acid (main product), ethanol, and lactic acid [TN 15.3].

I.2 - Growth on faecal material

The first MELiSSA test substrate for the liquefying compartment was rat faeces. The growth of *Cl. thermocellum* and *Cl. thermosaccharolyticum* on rat faces failed [TN 7.1], and the breakdown efficiency of proteins in rat rat faeces (fed with spirulines), observed with *Copronothermobacter proteolyticus I8*, [TN 15.4; TN 15.5] was very low whatever were the cultures conditions. In 1994, Demey and Van Menen led experiments with several other types of faeces:

- artificial human faeces (obtained from a shime reactor; liquid product). The dry matter of artificial human faeces was rather low (14 g/l) and there was not necessary to dilute them for experiments.
- pig manure (which can contain antibiotics).The dry mater is about 35g/l, then experiment did not require to dilute the pig manure.
- human faeces. The collected human faeces had a dry matter content of 20 to 35%. The faeces were diluted ten times with demineralised water in order to guarantee accurate determinations.

The objective was to test the capacity of the selected strains to degrade these substrates in axenic and coculture conditions. The experimental results and substrates compositions were well summarised in the final report for 1994 activity, and are reported in table 1. The degradation, of faeces in presence of urea or with ammonia addition were not performed during these closed bottles studies.

Substrate	Axenic culture	Non axenic culture *Cl. thermocellum* *C. proteolyticus*	Non axenic culture 3 selected strains
Artificial human faeces	No degradation occured	No considerable liquefaction and no biogas production Small ammonia production	No considerable liquefaction and no biogas production Small ammonia production
Artificial human faces + gelatine (proteic source)	No degradation occured	Small ammonia production	
Pig manure	No biodegradation excepted small amount of VFA produced by *Cl. thermocellum*	No considerable liquefaction and no biogas production	No considerable liquefaction and no biogas production
Pig manure + gelatine	No considerable liquefaction of manure Small amount of VFA by *Cl. thermocellum* Slight production of VFA by *C. proteolyticus I8.*		
Human faeces (autoclaved 20 min)			Autoclavation insufficient to have a complete death of all bacteria. Faeces broken down in blank and tests, then it is not possible to evaluate the capacity of the consortium to degrade the faeces.
Human faeces (autoclaved 40 min)			Biodegradation of human faeces, but not with the efficiency of autochtonous bacteria (two time lower).

Table 1: Review of the biodegradation experiments on different MELiSSA substrates for the liquefying strains selected. Experiments were performed in closed bottle.

I.3- The non axenic biodegradation

The results of biodegradation tests of human faeces by co-cultures of the selected strains have shown that in fact the autochtonous strains have a better liquefying efficiency than the selected ones. In TN 22.5, the results of batch reactor experiments for the biodegradation of human faeces by autochtonous bacteria were presented. These preliminary test proved that the use of autochtonous strains were the best choice for the anaerobic digestion of the crew's waste. Since 1996, all the works concerning the liquefying compartment were oriented to the optimisation of the anaerobic biodegradation of faeces (and cellulose and urea) by autochtonous strain in fed-batch bioreactors [TN26.1; TN 26.2; TN 26.3; TN. 34.1; TN 34.2; TN 34.3].

These studies concerns:

- the analysis of the steps of anaerobic biodegradation of organic material
- the characterisation of the composition of human faeces
- the optimisation of the analytical methods

- the study and the optimisation of the process conditions
- the study of pretreatments of the substrate
- the control and the removal of pathogenic strains

I.3.1 - Faeces degradation: the anaerobic steps

The multi-step process of anaerobic fermentation was described by Demey and van Meenen [TN 26.1; TN 26.2; final report for 1995 activity]. The 3 steps are illustrated by figure 1, and to each of them is associated a group of bacteria.

Step 1: hydrolysis and acidogenesis. The bacteria (acidogens) hydrolyse organic polymer into monomers, which are further broken down through oxidation-reduction processes, producing CO_2, H_2, and volatile fatty acids.

Step 2: acetogenesis. The bacteria (acetogens) break down VFA and alcohol into mainly acetate, CO_2 and H_2.

Step 3: methanogenesis. There are two major subgroups of bacteria. Those which cleave acetate into CH_4 and CO_2 (acetoclastic), and those which convert H_2/CO_2 into CH_4, and CO_2.

All the bacteria work together, the substrate of ones is the product of the other, which makes the processes difficult to analyse in details. By studying the end products of the biodegradation, it seems difficult to analyse the different steps separately.

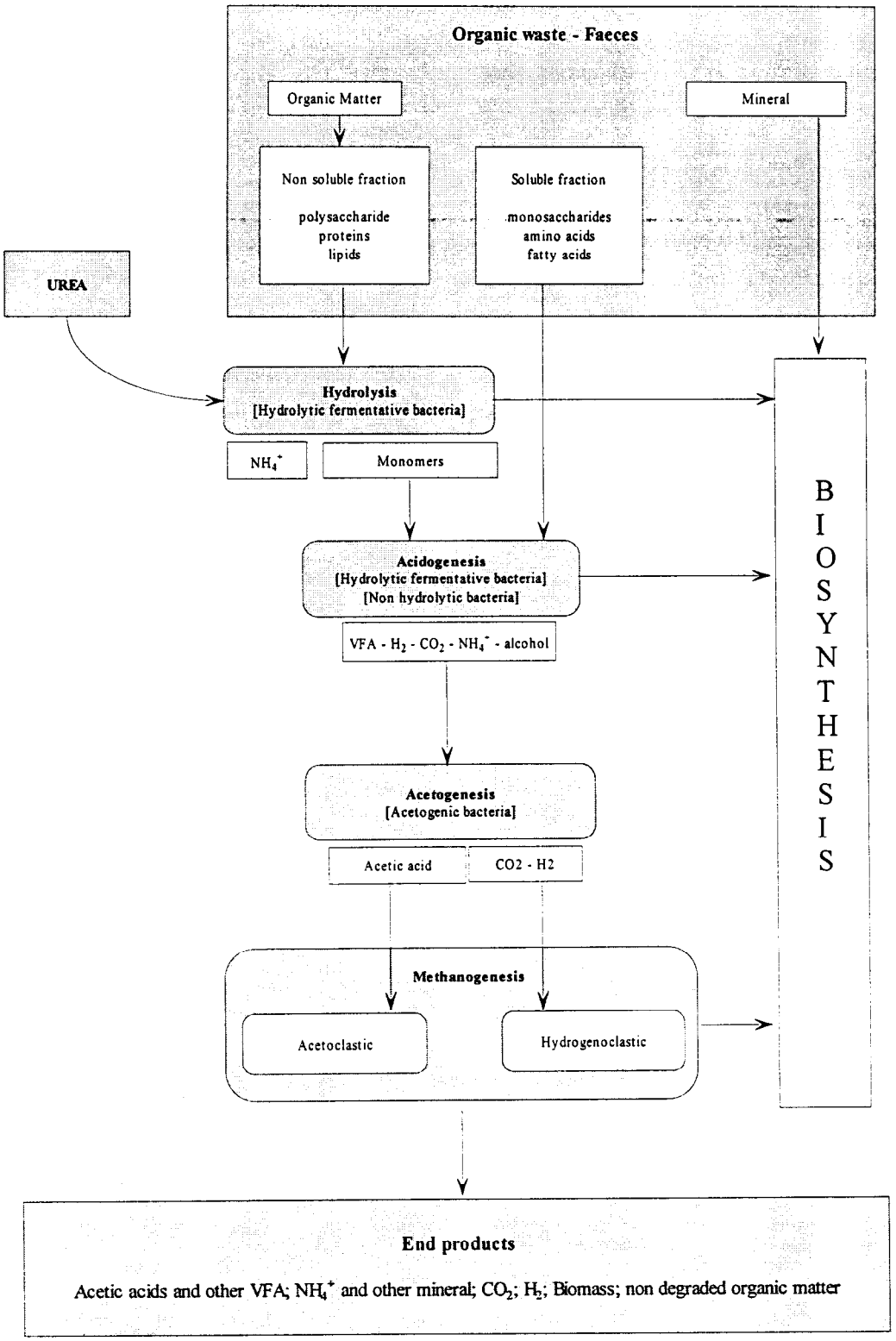


Figure 1: Steps of the anaerobic fermentation.

I.3.2 - Batch processes: objectives and main results

The present studies purchased by EPAS concerns the optimisation of the anaerobic process for the faeces biodegradation in reactors. The experiments led by EPAS with fed batch bioreactors are reviewed in table 2.

Reactor	From previous reactor	Vol. load [g OM/d.l.HF]	Recycle	water/HF ratio	MRT [days]	Temp. [°C]	pH	Dry matter [%]	Exp. period [days]	Inoculum	Substrates	Inhibition methano.	Ref.
RI-1	-	5.7	-	-	25	55	7	12.9 ± 0.3	53	Auto.	HF	No	TN26.1 TN26.2
RI-2	RI-1	5.8	-	1.75	12	55	6.7	5.2 ± 0.4	23	Auto	HF	No	TN26.1 TN26.3
RI-3	RI-2	5.1	38	2.70	∞	55	6.8	5.2 ± 0.4	20	Auto	HF	No	TN26.3
RI-4	RI-3					55		5.2 ± 0.4		Auto	HF	No	
RII-3	RI-2	4	35	4.7	∞	55	7.3	2 ± 0.3	20	Auto Ino. Cel.	HF	No	TN26.3
RIII-4	-	4	35	4.7	∞	32	7	2 ± 0.4		Auto	HF	No	TN26.3
RIV-4	RI-3				∞	55		5.2 ± 0.4		Auto	HF	No	
TI P1: 0-14 d. P2: 14-29 d. P3: 29-50 d.	RI-4	Variable	-	-	Variable	55	8-8.5		50	Auto	 P1: cl+HF P2: cb P3: cl	No	TN34.1 TN34.2
TI+Cl P1: 0-14 d. P2: 14-26 d. P3: 26-50 d	RI-4	Variable	-	-	Variable	55	8-8.5		50	Auto	 P1: cl+HF P2: cb P3: cl	No	TN34.1 TN34.2
TRc/TRcf P1: 0-42 d. P2: 42-61 d.	TI	 P1: 1g cl/d. P2: variable	-	-		55	8-9		61	Auto	 P1: cl P2: cl+HF	No	TN34.2
TRci/TRcf P1: 0-42 d. P2: 42-61 d.	TI	 P1: 1g cl/d. P2: variable	-	-		55	8-9		61	Auto	 P1: cl P2: cl+HF	Yes UREA	TN34.2
TRc P1: 0-42 d. P2: 42-61 d.	TI+Cl	 P1: 1g cl/d. P2: variable	-			55	8-9		61	Auto Clost	 P1: cl P2: cl	No	TN34.2
TRci P1: 0-42 d. P2: 42-61 d.	TI+Cl	 P1: 1g cl/d. P2: variable	-			55	8-9		61	Auto Clost	 P1: cl P2: cl	Yes UREA	TN34.2
TR_FM	TRc/TRcf	variable	Yes ?	200ml/20g wet	variable	55	8		28	Auto	HF	No	TN34.3
TR_FMsoni	TRc/TRcf	variable	Yes ?	200ml/20g wet	variable	55	8		28	Auto	HF sonic.	No	TN34.3

Table 2 (to continue)

Reactor	Objectives - (Remarks)	Parameters studied	Mains results
RI-1	Biodegradation of non diluted HF by thermophilic autochtonous strains	Dry matter; OM Organic N; Ammonia VFA and biogas pH	Continous study of parameter evolution. Mass balance calculation based on Buswel equation. Calculation of breakdown efficiencies (60 to 90%). Unable to centrifuge reactor content. PH very stable (reactor strongly buffered) as the other parameters studied (excepting the soluble OM content) during the experiments. In further experiments only yields are then reported. The VFA profile of reactor RI-1, RI-2 and RI-3 are quite identical
RI-2	Idem RI-1 with diluted HF (Only mean yields are reported as results)	Idem RI-1	Comparison of yields between RI-1, RI-2, RI-3 RII-3 and RIII-4. Centrifugation to separate soluble and non soluble compounds succeed. The VFA profile of reactor RI-1, RI-2 and RI-3 are quite identical
RI-3	Idem RI-2 with recycling of non soluble compounds (Only mean yields are reported as results)	Idem RI-1	Comparison of yields between RI-1, RI-2, RI-3 RII-3 and RIII-4. Recycled material is slower biodegraded than new fed HF. Proteins are not accumulated and better degraded than the other substrates. The VFA profile of reactor RI-1, RI-2 and RI-3 are quite identical
RI-4	Continuation of reactor RI-3		Transitory process. This reactor was used as the precursor for the further batch experiments.
RII-3	Idem RI-3 with addition of a cellulolitic inoculum to improve HF breakdown. (Only mean yields are reported as results)	Idem RI-1	Comparison of yields between RI-1, RI-2, RI-3 RII-3 and RIII-4. Particulate OM stays high. VFA content greatly decreases, the inoculum has power up the methanogenesis in fact. Propionate is the major VFA. This must be compared with the Trcf reactors. Usually, propionate is considered as an indicator of a process problem.
RIII-4	Idem RI-3 but in mesophilic growth condition (37°C). (Only mean yields are reported as results)	Idem RI-1	Soluble OM is increased. Degradation of carbohydrates was more efficient but degradation of proteins is less efficient than in thermophylic process.
RIV-4	Idem RI-3 and addition of urea to inhibit methanogenesis.		
TI	Biodegradation of cellulose by thermophylic bacteria	Gas production VFA pH Biodegradation efficiency	Final biodegradation efficiency:61% VFA consist mainly in propionic acid At day 50 great decrease of VFA, even with the addition of new substrate to degrade, but production of biogas continue. pH very stable
TI+Cl	Biodegradation of cellulose by thermophylic inoculum,, *Cl thermocellum* and *Cl thermosaccharolyticum*.	Idem TI	Final biodegradation efficiency:57% VFA consist mainly in propionic acid At day 21 great decrease of VFA. The 21 first days can be considered as a lag phase. Clostridia strains enhance the the acidogenesis and the acetogenesis steps.

			pH very stable
TRc/TRcf	Control reactor for the study of inhibition of methanogenesis by urea (reactor TRci/TRcf) HF was pretreated (acidification in a reactor during 4 days)	VFA Total biogas and methane Gas conversion and degradation efficiency pH Amonium	No inhibition of methanogenesis. Conversion efficiency of cellulose between 54% and 68% Preacidification treatment has no influence on biogas composition Acetic acid is the main VFA, excepted after the first addition of HF.
TRci/TRcf	Inhibition of methanogenesis by addition of urea. HF was pretreated (acidification in a reactor during 4 days)	Idem TRc/TRcf	Variation of pH (buffer capacity not sufficient to ensure stability during VFA production and urea hydrolysis). Hydrolysis of urea stimulated by addition of HF. Acetic acid is the main VFA. In inhibitory conditions, HF is not degraded, and acidogenesis and methanogenesis are both inhibited.
TRc	Control reactor for the study of inhibition of methanogenesis by urea (reactor TRci/TRcf). Substrate is only cellulose.	Idem TRc/TRcf	This process occurs in the conditions of the TI reactor, and can be compared to it. At
TRci	inhibition of methanogenesis by addition of urea. Substrate is only cellulose.	Idem TRc/TRcf	pH variations. Both inhibition of acetogenesis and methanogenesis
TR_FM	Control reactor for the experiment on sonication of faeces before feeding.	OM composition Biogas and methane production VFA	Biodegradation of OM elements. is calculated
TR_FMsoni	Study of the effect of a preatreatment of faeces by sonication.	Idem TR_FM	No effect on the biodedradation efficiency?

Table 2: Overview of the fed batch experiments leaded by EPAS since the last years.

In Cl: Inoculum from an vegetable wastes digester (cellulolitic)
Auto: autochtonous strains
Clost: clostridia inoculum (selected strains of Cl thermocellum, CVl. Thermosaccharolyticum; Copronobacter proteolyticus)
Pi: time periode i
HF: Human Faeces
cl: cellulose
cb: cellobiose
MRT: Mean Residence Time

From these experiments, it was concluded that

1 - The thermophilic growth conditions are preferred for the elimination of pathogens. In thermophilic conditions (55°C) the proteins biodegradation is only slightly enhanced, compared to the mesophilic (37°C) conditions.

2 - The addition of the selected clostridia strains (*Cl. thermocellum* and *Cl. thermosaccharolyticum*) enhances the first steps of biodegradation, but the total biodegradation efficiency seems unchanged. The use of a vegetable waste digester inoculum accelerates the methanogenesis step;

3 - The treatment of faeces by pre-acidification or by sonication does not increase the biodegradation efficiency;

4 - The recycling of non degraded faecal material can be made only by diluting the feed, but the recycled material is degraded slower than the fresh feed;

5 - For quite all processes, pH is very stable (7-8.5), indicating that the medium is well buffered;

6 - The methanogenesis is inhibited by the addition of urea, but this inhibits too the other steps of anaerobic degradation and then reduces the degradation efficiency.;

7 - With human faeces, the main VFA produced during acidogenesis seems to be propionate, while with cellulolytic material it is acetate.

8 - The odorous gas produced probably contains corrosive compounds such as NH_3 or H_2S. The composition of the gas produced is one point to investigate.

It can be noticed that several closed bottle tests were performed for the study of inhibition of methanogenesis by ammonia and the biodegradation efficiency of cellulose. The removal of pathogens was also studied.

I.4 - Process efficiencies and yields reported

From the different experiments (fed batch reactors and closed-bottles), conversion efficiencies and mass balance could be calculated.

Detailed biodegradation efficiency (Closed-bottle tests-Reactors RI-1 to RIV-4)

A mass balance representation and a biodegradation efficiency based upon analytical measurement of organic matter have been already proposed (yearly report, 1994). The principle is exposed below.

The dry matter is composed of organic matter and ash:

$$DM=OM+Ash$$

The organic matter is made of proteins (PROT), lipids (LIP), carbohydrates (CARB) and VFA.

$$OM=PROT+LIP+CARB+VFA$$

After biodegradation, new compounds are present as biogas forms (BIOGAS). The mass balance for the process is written (i and e are respectively for the beginning and the end of the process).

$$OM_i=OM_e+BIOGAS_e$$

Using these relations, some non-measured compounds can be estimated (as the lipids+carbohydrates content). With the analysis of the organic matter at the beginning and at the end of the process, detailed conversion efficiencies can be calculated. The biomass content can be taken into account assuming that (Yearly report 1994):

- the initial biomass represents 1/3 of the total OM
- the biosynthesis is 0.2mg biomass/mg OM degraded

Some detailed biodegradation efficiencies are reported in table 3. This method for efficiencies calculation was used only in the early mass balance descriptions of the process.

Biodegradation efficiency from VFA and biogas (Reactors TI to TRci)

For the calculation of the conversion efficiencies, we first made a mass balance representation of the process. The mass balance calculations were based upon the Hill equations reported by Angelidaki et al. (1993) and adapted to the liquefying compartment by Demey [TN 26.3].

From Angelidaki, it was calculated that:

For a complete process

1g OM → 0.153g biomass
0.907g biogas

For a methanogenesis inhibited process (up to acetogenesis)

1g OM → 0.122g biomass
0.755g VFA
0.194g biogas

The problem was simplified by Demey [TN34.1] and it was assumed that:

1g VFA → 1g OM converted
1g biogas → 1g OM converted

Then the conversion efficiency was calculated as:

$$\text{efficiency} = \frac{\text{Converted OM}}{\text{feed OM}} = \frac{[\text{VFA} + \text{Biogas}]}{\text{feed OM}}$$

Some biodegradation efficiencies are reported in table 3. This method for the process efficiency calculation is the current method used.

Other biodegradations efficiencies

The efficiencies calculations used in EPAS experiments can be used for a complete anaerobic process in which the objective is to degrade organic matter into biogas (CO_2 and methane). In

the MELiSSA loop, methanogenesis must be inhibited. The main objective is the production of VFA (acetate), CO_2 and to lower extend H_2, which can be used by the following compartments. In order to determine the process efficiencies, the following parameters are proposed:

1 - The VFA production efficiency, defined as:

$$\frac{\text{VFA produced}}{\text{Dry matter or organic matter fed}}$$

The dry matter includes ash, organic matter and VFA. This ratio can be important for the design of the photoheterotrophic compartment II.

2 - The CO_2 production efficiency

$$\frac{CO_2 \text{ produced}}{\text{Dry matter or organic matter fed}}$$

The CO_2 production is important for the photosynthetic compartiment

3 - The Hydrogen efficiency

$$\frac{H_2 \text{ produced}}{\text{Dry matter or organic matter fed}}$$

It is important to determine the H_2 production. This compound can only be assimilated by the photoautotrophs of compartment II.

4 - The N-balance of the process

$$\frac{NH_3}{\text{N - organic fed}}$$

6 - The Mineral balance of the process

$$\frac{\text{Mineral in liquid outlet}}{\text{Mineral in liquid inlet}}$$

The mineral balance will be one of the point to investigate for the complete loop. A mineral balance would be calculated for each of the MELiSSA compartment. For simplicity, the mineral balance is based here only on the liquid mineral content (minerals include in biomass or solid wastes of the liquefying compartment are not include), assuming that only mineral in the liquid can be used in biological reactions.

6 - The global efficiency of the process

It can be difficult to determine the global efficiency of the process. One way is to use a combination of the efficiency for the main elements (Carbon Nitrogen and Mineral):

$$\frac{\text{Mineral in liquid outlet}}{\text{Mineral in liquid inlet}} \cdot \frac{\text{C - (VFA + CO2) produced}}{\text{C - (Dry matter or organic matter fed)}} \cdot \frac{N - NH_3}{\text{N - organic fed}}$$

Substrate / Reactor	Closed-bottle tests	Faeces (diluted or not) RI-1	TR_FM	TR_FMsoni	TRc/TRcf	TRci./TRcf	Cellulolitic TI	TI+CL	TRci
OM degradation									
Total	35?	46	37	38	44-62	fall to 20	60	57	35-45
Non bacterial	53?	70	55	57					
Total proteins	47	60	51	51					
Total lipids+carb.	30	40	29	31					
Non bacterial Prot.	89	88	76	76					
Non bacterial lip+carb.	57	60	43	46					

Table 3: Conversions efficiencies reported for several experiments led at EPAS. Fed batch and closed bottle tests.

II - Mass balanced description of biodegradation stages

II.1 - Some observations and remarks about anaerobic digestion.

First, as for all bioprocesses, the products formed (qualitatively and quantitatively) depend both on the type of microorganisms and of the type of substrate used. This can be illustrated by experiments TI and TI+Cl, where the addition of clostridia slightly enhances the assimilation of VFA.

The carbon and energetic metabolism of anaerobic fermentative bacteria have been well studied (Gottschalk, 1985). Fermentative anaerobes carry out a variety of oxidation-reduction reactions, involving organic compounds, carbon dioxide, molecular hydrogen and/or electron transport phosphorylation. The ATP yield (mol ATP/mol substrate consumed) is very low. Therefore, a large amount of fermentation end-products is formed.
As the substrate used in the MELiSSA loop is complex (faeces) and the bacteria colonizing the compartment are autochtonous bacteria (i.e. a consortium of unknown strains), it can be useful to review the different possibilities of anaerobic biodegradation. Most of the informations cited here are taken from the book "bacterial metabolism" (Gottschalk, 1985).

II.1.1 - Anaerobic metabolism

II.1.1.1 - Alcohol fermentation

Carbon metabolism

Alcohol fermentation is usually the domain of yeasts. Nevertheless some bacteria are able, and are used (as *Zymomonas mobilis*) to produce alcohol, from sugar or starch, for industrial purpose. Two pathways can be observed for ethanol production by bacteria:

1 - If pyruvate carboxylase is present (generally rare in bacteria)

$$\text{Glucose} \longrightarrow 2\ \text{pyruvate} \xrightarrow{\text{pyruvatede carboxylase}} \begin{matrix} 2\ \text{ethanol} \\ 2\ CO_2 \end{matrix}$$

2 - In other cases, acetyl-CoA functions as ultimate precursor for acetaldehyde and is reduced by acetaldehyde deshydrogenase.

$$\text{Glucose} \longrightarrow \begin{matrix} 2\ \text{acetylCoA} \xrightarrow{\text{acetaldehyde deshydrogenase}} 2\ \text{acetaldehyde} \longrightarrow 2\ \text{ethanol} \\ 2\ CO_2 \end{matrix}$$

Gottschalk notes that some thermophiles such as *Cl. thermohydrosulfuricum, Thermoanaerobium brockii* or *Thermobacter ethanoliticus* are developed to produce ethanol but they excrete in addition large amounts of acetate and lactate.

Energetics

The net ATP yield of the alcohol fermentation is 2ATP/mol glucose, much lower than the ATP yield of aerobic metabolism. The reaction Glucose→ethanol does'nt produce $NADH,H^+$. It can be noticed too that the acetyl-CoA is not oxidised by the tricarboxylic acid cycle (Gottschalk, 1985). The cycle is interrupted between α-oxoglutarate and succinyl-CoA.

II.1.1.2- Lactate fermentation

Carbon metabolism

Lactate is a very common product of bacterial fermentation. Some genera -often called lactic acid bacteria- form large amounts of this product. These micro-organisms have in common that they are highly saccharolytic and that they lack most anabolic pathways.
For the fermentation of carbohydrate to lactate, 3 pathways are employed.

The homofermentative pathway (*L. lactis, S. faecalis*).

Glucose $\longrightarrow$ 2 lactate

The heterofermentative pathway.

Glucose $\longrightarrow$ lactate + ethanol + CO_2

The bifidum pathway.

2 Glucose $\longrightarrow$ 3 acetate + 2 lactate

Of course, some other sugars other than glucose are fermented by lactic acid bacteria. It can be noticed that with fructose, mannitol can be produced. Some other fermentation can be associated to the lactic acid production such as the malo-lactic fermentation (mainly for wine). Other products can be excreted with lactate, as diacetyl and acetoine, but this concerns mainly the fermentation of milk.

Energetics

Both heterofermentative and homofermentative pathways have the same ATP/glucose yield equal to 2. As for alcohol fermentation, no NADH,H+ are formed through this reaction. The bifidum pathway has a quite higher ATP yield (2.5 ATP/glucose).

II.1.1.3 - Butyrate and butanol-acetone

The genera *Clostridium, Butyrivibrio, Eubacterium* and *Fusobacterium* carry out this type of fermentation. The clostridia employ the phosphotranspherase system for sugar uptake and the Embden-Meyerhof-Parnas pathway for degradation of hexose phosphates to pyruvate. The conversion of pyruvate to acetyl-CoA involves an enzyme system, the pyruvate-ferredoxine oxidoreductase. This system converts pyruvate to acetoacetyl, CO_2 and produces H_2. At pH 7, the redox potential of ferredoxin is the same as that of the hydrogen electrode, what allows to transfer electrons to hydrogenase even in an environment saturated with H_2.

$$\text{Glucose} \longrightarrow 2\ \text{pyruvate} \xrightarrow{\text{pyruvate-ferredoxin transferasesystem}} \begin{array}{l} 2\ H_2 \\ 2\ CO_2 \\ \text{acetoacetyl - CoA} \longrightarrow \text{butyrate} \end{array}$$

Some butyrate producing clostridia form small amounts of n-butanol and acetone. Few species can shift from butyrate production to this solvent production.

Energetics

The ATP yield on glucose is 3 ATP for the butyrate fermentation. No NADH,H+ is produced.

II.1.1.4 - Mixed acid and butanediol

This type of fermentation is carried out by enterobacteria (genera *Escherichia*, *Salmonella* and *Shigella*). These organisms ferment sugars to lactic, acetic, succinic and formic acids. In addition CO_2 and H_2 are formed. For all organisms, hexose are broken down by Embden-Meyerhof-Parnas pathway.

The pathway leading to succinate branches off at phosphoenolpyruvate.

All other end products are derived from pyruvate, following the Embden-Meyerhof-Parnas pathway (3 enzymes systems, the activity of which determines the quantity of products formed).

The 3 enzyme systems are:

1 - the lactate dehydrogenase

2 - the pyruvate-formate lyase. It functions only in anaerobic conditions, and the reaction is irreversible. Its advantage over the pyruvate dehydrogenase complex used in aerobiose is that the formation of acetyl-CoA is not accompanied by the reduction of NAD. The formate produced can be further used by the formate-hydrogen lyase. This enzyme system is membrane-bound and transfers electron to

-nitrate reductase if nitrate is present
-fumarate reductase if fumarate is present
-hydrogenase in the other cases, producing H_2.

3 - the α-acetolactate synthase. This enzyme is active under slightly acidic conditions (pH 6 enzyme). It is involved in the 2,3-butanediol formation.

II.1.1.5 - Propionate and succinate

Propionate is one of the major end products of fermentation. Many organisms ferment glucose to propionate, acetate and CO_2:

$$1.5\ \text{glucose} \longrightarrow 2\ \text{propionate} + \text{acetate} + CO_2$$

A preferred substrate of propionate-forming bacteria is lactate, so that these organisms can growth with the major end product of lactate fermentation. From lactate 2 pathways are related:

1- the acrylate pathway. It occurs only in a few organisms.

$$3\ \text{lactate} \longrightarrow 2\ \text{propionate} + \text{acetate} + CO_2 .$$

2- the succinate-propionate pathway.

$$\text{lactate}\ NADH + H + ADP + Pi \longrightarrow \text{propionate} + NAD^+ + ATP + 2\ H_2O$$

II.1.1.6 - Acetate fermentation by acetogenic bacteria

Acetogenic bacteria can grow using H_2+CO_2 or sugar, by producing acetate as the most non gaseous metabolite:

$$\text{Glucose} \longrightarrow 3\ \text{acetate}$$

$$4\,H_2 + 2\,CO_2 \longrightarrow \text{acetate} + 2\,H_2O$$

II.1.1.7 - Methane fermentation

Methane is the most reduced organic compound and its formation is the terminal step of anaerobic processes. Methanogenesis is the domain of archeobacteria. As previously noted, 2 groups of bacteria can be observed: those using C1 substrates (CO_2+H_2; formate, methanol, methylamines) and those using a C2 substrate (acetate). Complex organic substrates cannot be utilised by methanogens.

$$\text{Acetate} \longrightarrow CH_4 + CO_2$$

$$4\,H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O$$

II.1.1.8 - Amino acids fermentation

Sugar and organic acids are not the only substrates for anaerobes. Amino-acids (formed from proteins degradation by extracellular proteases) and purine/pyrimidine bases can be used as carbon substrate and nitrogen substrate.

Single amino acids can be fermented through specific pathways. As an example, alanine is fermented by *Cl. Propionicum* via the acrylate pathway.

Although a number of clostridia species grow on a single amino acid, many clostridia prefer to ferment mixtures of amino acids. They carry out coupled oxidation-reductions between pairs of amino acids. The reactions involved are called Stickland reactions.

II.1.1.9- Membrane energisation and organic acids

The membrane of anaerobe has to be energised like that of aerobes. This means that protomotive force must be continuously generated. Some observations were made on the membrane energisation for anaerobes

1 - It exists an uncoupling effect of organic acids on the ATP synthase
2 - It exists a coupling of lactate exportation with H^+ translocation.

This uncoupling exists only at low pH values (3 - 4).

II.1.1.10 - Degradation of organic matter to CH_4,CO_2 and minerals

It was seen that methanogens can only use some C1 and acetate. The end products of fermentation of glucose or complex substrates must be themselves reduced to CO_2 and acetate.

Lactate can be converted by propionibacteria (cf. II.1.1.5). For the other compounds, the reactions involved present a free energy positive, and then they will not proceed except under condition in which a product is kept at very low concentration.

$$\text{ethanol} + H_2O \longrightarrow \text{acetate} + H^+ + 2\,H_2 \qquad \Delta G^{0'} = 9.6\ \text{kJ}$$

$$butyrate + 2H_2O \longrightarrow 2\ acetate + H^+ + 2\ H_2 \qquad \Delta G^{0'} = 11.5\ kJ$$
$$propionate + 3H_2O \longrightarrow acetate + H^+ + bicarbonate + 2\ H_2 \qquad \Delta G^{0'} = 76.1\ kJ$$

The high affinity of methanogens towards H_2 keeps the partial pressure of H_2 below 10^{-4} atm. This is low enough to allow the formation of H_2 from $NADH,H^+$ and from the above substrates. This coupling between acetogens and methanogens is a syntrophic association called "interspecies hydrogen transfer".

II.1.1.11 - Anaerobiosis vs. aerobiosis

The mean biomass formed during anaerobic processes is not significantly different of the one produced in aerobic processes (Roels, 1983):

Average biomass from aerobic cultures: $CH_{1.83}O_{0.56}N_{0.17}$

Average biomass from anaerobic cultures: $CH_{1.80}O_{0.55}N_{0.18}$

Comparing the use of thermodynamic relations and tabulated data for the calculation of the free enthalpy of anaerobic and aerobic processes, Roels (1983) showed the importance of the "chemical entropy" dissipation as the driving force in anaerobic processes.
If the thermodynamic efficiency of growth is slightly better in anaerobic culture (70% for glucose as substrate) than in aerobic culture (64% for glucose as substrate), the biomass yield is much lower (table 4).

Energy-generating reaction	Yx/s (Cmol biomass/Cmol substrate)
Glucose→ethanol+CO2	0.14
Glucose→lactate	0.13-0.24
Glucose→2/3 propionate + 1/3 acetate + CO2	0.238
Glucose→0.36CH4+ 0.64 acetate + CO2	0.260
Acetate→methane+CO2	0.057
Methanol→methane+CO2	0.043
Formate→methane+CO2	0.043
Propionate→methane+CO2	0.077

Table 4: Growth yields for anaerobic growth without external electron acceptors. From Roels (1983). On glucose, the mean growth yield is 0.56 Cmol biomass/Cmol substrate, in aerobic conditions.

II.1.2 - Conclusion-summary

It appears that for a single substrate, as glucose, different end products, on the qualitative and quantitative point of view, can be obtained. This makes hard to predict the evolution of the anaerobic degradation of the substrate by a consortium of unknown bacteria. The problem is made more difficult by the use of a complex substrate. **It can be concluded to the current impossibility to use a structured approach for the representation of anaerobic degradation of faecal material by autochtonous bacteria.**

The energetics of the growth under anaerobic conditions appears to be sensible to the growth conditions (partial presure of H_2), and the energisation of the membrane involves several enzyme complex, membrane-bounded, (fumarate reductase, H_2 dehydrogenase, Nitrate reductase, NADH dehydrogenase), coupling and uncoupling with substrate/products intake or excretion.

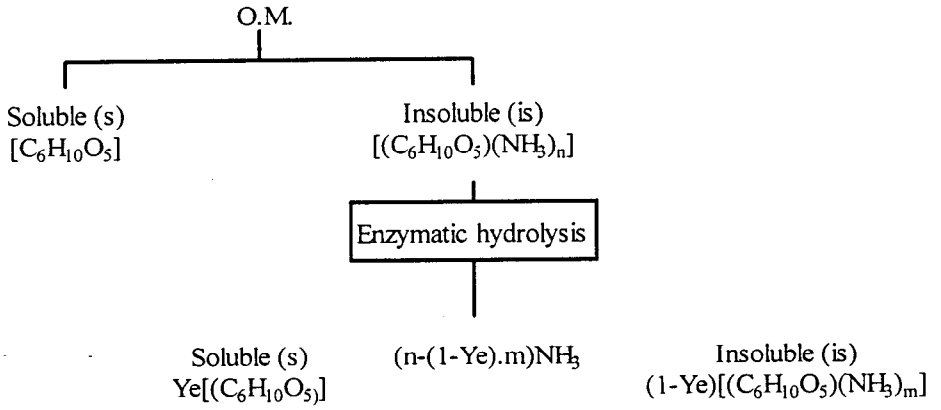
In the complete anaerobic process, from organic matter to CH_4, it exists an interspecies hydrogen transfer, keeping low the H_2 partial pressure. If the partial pressure of H_2 is greater than 10^{-4}, the acetogenic step cannot work. But the degradation of glucose can continue, accompanied with H_2 production (see butyrate fermentation), if the organism uses the pyruvate ferredoxin oxidoreductase complex.
The growth yield in anaerobic processes is very low.

II.2 - Some models found in literature

II.2.1 - Model of Hills-Angelidaki

The model proposed by Angelidaki et al. (1993) was developed from a previous model of Hills. Their model was used by Demey et al. (cf. I.4) for the calculation of the efficiency of the organic matter biodegradation.

Principles of the model.
The model was developed in order to be adapted to various complex substrates. The substrate is described as an insoluble (is) and a soluble part.



n represents the Nitrogen content of the substrate and m the part of the Nitrogen element released as NH_3 by hydrolysis.
The adjustment of n and of the ratio of soluble/insoluble part allows the representation of different types of substrates. The enzymatic hydrolysis involves 2 other parameters: Ye (relative to the fraction of soluble matter obtained) and m (relative to the NH_3 released). This model allows the representation of different substrates and processes.

The different steps of the methanogenesis are represented by a set of equations. The biomass production is included in these equations. The CHONSP formula of $C_5H_7O_2N$ (i.e. $CH_{1.4}O_{0.5}N_{0.2}$) used is quite different of the mean formula proposed by Roels (1983) for

anaerobic micro-organisms ($CH_{1.8}O_{0.5}N_{0.2}$). The mass balanced equation of Angelidaki et al. are reported in table 6.

Dynamic model of Angelidaki

These mass equations were associated to a biological kinetic model and a physical model (gas-liquid equilibria, pH and temperature). The kinetic parameters used can complete those reported by Demey (TN 26-1).

Remarks:

1 - The H_2 producing equations (acetogenic step) have been compiled with the H_2 consuming equation. This is justified by the interspecies hydrogen transfer.

2 - The representation of organic wastes to degrade is of interest and allow an elemental representation of organic wastes, when it was only defined by its mass. It presents the inconvenience to be less reliable if an elemental composition of the waste has been previously established.

3 - The dynamic model equation did not include the biomass terms. Moreover the biomass (inoculum) setup was not described.

II.2.2 - Model of Dunn

Dunn et al. (1992) proposed a dynamic model for whey anaerobic degradation with a five organisms process (five equations).

Principle

A single equation is used to represent the hydrolysis and the acidogenesis step of whey anaerobic degradation.

$$\text{Whey} \longrightarrow 0.5\ \text{butyrate} + 0.12\ \text{propionate} + 0.15\ \text{acetate} + 0.23\ CO_2 + 0.24\ H_2$$

The acetogenic step is represented by the 2 equations

$$\text{butyrate} + 2\ H_2O \longrightarrow 2\ \text{acetate} + 2\ H_2$$

$$\text{propionate} + 2\ H_2O \longrightarrow \text{acetate} + 3\ H_2 + CO_2$$

The methanogenic step is represented by:

$$\text{acetate} \longrightarrow CH_4 + CO_2$$

$$4\ H_2 + CO_2 \longrightarrow CH_4 + 2\ H_2O$$

Dynamic modelling

The dynamic modelling of anaerobic process developped by Dunn et al. is different from that of Angelidaki et al.. Dunn calculates the growth of each of the organism and then deduces the substrates consumption and the products formation. The coupling between catabolism (equations written above) and biomass formation appears only in the dynamic model. It is stated that:

0.03 C-mol biomass is produced by Cmol of whey consumed

0.05 C-mol biomass is produced by Cmol of butyrate consumed
0.04 C-mol biomass is produced by Cmol of propionate consumed
0.05 C-mol biomass is produced by Cmol of acetate consumed
0.25 C-mol biomass is produced by Cmol of CO_2 consumed

As for the dynamic model of Angelidaki, the gas liquid and pH equilibria are taken into account.

Remarks

1 - The model of Dunn takes only into account acetate, butyrate, propionate, CO_2, H_2, CH_4 and biomass. There is then no complete mass balance of the process. Moreover, it seems that the substrate involved in the biomass synthesis is not taken into account.
2 - The whey composition is not defined, and it's degradation is not mass balanced.
3 - The equilibria for organic acids are accounted by an arbitrary "equilibrium factor".

II.3 - Present mass balanced modelling of the liquefying compartment activity.

At the present time no dynamic model was developed for the liquefying compartment. From past knowledge of the process, a mass balanced model has been developed to be used with the ProSim MELiSSA simulations (TN32.3).
The model is composed of 12 equations (table 5):

3 for the hydrolysis and acidogenic step
7 for the acetogenic step (one for each VFA considered i.e. propionate, butyrate, isobutyrate, valerate, isovalerate, caproate and isocaproate).
2 for the methanogenesis step

The organic wastes (faeces) are defined as a matter composed of proteins, carbohydrates and lipids, the relative composition of which is calculated in the previous compartment (crew) as a function of the food metabolised.
The protein composition of faeces (elemental formula and amino acids composition) was assumed to be identical of that of *Rs. capsulata* proteins composition in absence of other informations. From the knowledge of the end product of each amino acid degradation via the Stickland pathway (Andreessen, 1985 - Barraud et al.,1992), a complete stoichiometric equation for the hydrolysis and degradation of proteins was established, leading to the 8 VFA considered in the process.
The carbohydrates are assumed to be an hexose polymer, the hydrolysis of which leads to mono-hexose (i.e. glucose), and is degraded to acetate.
The lipids are assumed to be linoleic acid, and are degraded to acetate.

The biomass biosynthesis is not taken into account. I was unable to determine a coupling between the catabolic reactions and the biomass synthesis.

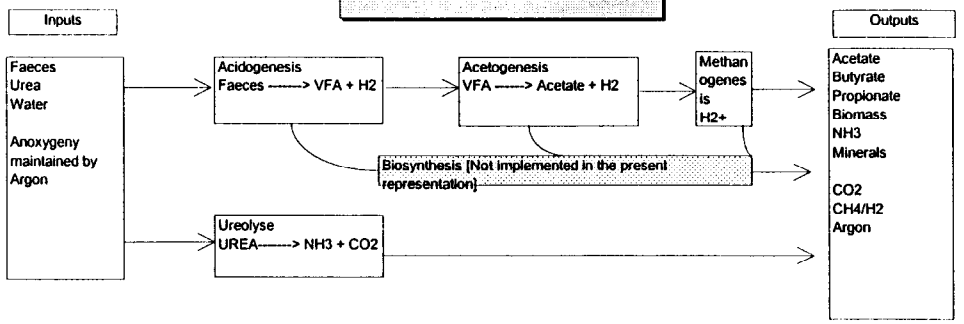
A $13^{th.}$ equation is added to take into account the fibre part, which was introduced when the plants where included in the food. Fibres are not digested and their elemental composition is calculated in the HPC compartment (TN32.3). Because of the variability of the composition

Liquefying - Human waste degradation - Mass balance description

Objectives: Solve the system Faeces ------> VFA + Minerals + GAS
Solve the system Ureae ------> CO2 + NH3

Fixed Data :
- % of faecal proteins degraded
- % of faecal lipids degraded
- % of faecal carbohydrates degraded
- % of urea degraded
- % of methanogenesis
- Fibre assimilated to carbohydrate
- Complete acetogenesis only for caproate, Isocap,,Valerate, Isoval,
- All ather VFA reduced to Acetate, Butyrate and Propionate
- No biomass production (lack of coupling factors)

Process Resolution



Fixed Stoichiometries

	Prot	Polyose	Lip	CH4	H2	H2O	Co2	Acet	Prop	Buty	IsoButy	Val	IsoVal	Cap	IsoCap
Hydrolysis + Acidogenesis of protei	-1				0,3406	-0,7142	-0,2527	-0,1520	-0,0125	-0,0151	-0,0076	-0,0125	-0,0177	-0,0099	-0,0173
Hydrolysis + Acidogenesis of polyoside		-1			0	-0,1667		0,5							
Hydrolysis + Acidogenesis of lipids			-1		1,375	-0,875	0	0,5							
Acetogenesis of propionate					3	-2	1	1	-1						
Acetogenesis of butyrate					2	-2	0	2		-1					
Acetogenesis of Isobutyrate					6	-4	2	1			-1				
Acetogenesis of valerate					5	-4	2	2				-1			
Acetogenesis of isovalerate					5	-4	1	2					-1		
Acetogenesis of caproate					4	-4	1	2						-1	
Acetogenesis of isocaproate					8	-6	2	2							-1
Methanogenesis CO2				1	-4	2	-1	0							
Acetoclastic methonogenesis				2	-4	2		-1							

Variable Stoichiometries

Acidogenesis of Fibre: Internal resolution of the stoichiometry Fibre -----> CO2 +Acetate + Mineral + H2
Function of the CHONSP compostion of fibre (itself function of the food ingested)

Table 5: Present description of the MELiSSA liquefying compartment for the ProSim-MELiSSA mass balance simulations

of the fibres, the coefficients of the equations cannot be fixed as for the 12 other equations, and are then calculated on the basis of:

$$[CHONSP]_{Fibres} + H_2O \longrightarrow CO_2 + Acetate + H_2SO_4 + H_3PO_4 + H_2$$

The overall description of the mass balanced model for the liquefying compartment is given in table 5.

II.4 - Development of a model for steady state (ProSim-MELISSA loop modelling) and dynamic modelling.

II.4.1 - Mass balanced models

Two mass balances are proposed.

The first is in fact the set of equations of the Angelidaki-Hills model, always used by Demey for the calculation of the process efficiency. The equation of this model includes the biomass biosynthesis. The model is made of 6 equations, listed in table 6.

Hydrolysis	$[C_6H_{10}O_6.nNH_3]_{is} \longrightarrow Ye[C_6H_{10}O_5]_s + (1-Ye)[C_6H_{10}O_5.mNH_3] + (n-(1-Ye).m)NH_3$
Acidogenesis	$[C_6H_{10}O5]s + 0.1115NH_3 \longrightarrow 0.1115C_5H_7O_2N + 0.744Acetate + 0.5Propionate + 0.49Butyrate + 0.6909CO_2 + 0.0254H_2O$
Acetogenesis	$Propionate + 0.0458NH_3 + 1.764H_2O \longrightarrow 0.0458C_5H_7O_2N + 0.9345Acetate + 0.902CO_2 + 2.804H_2$
	$Butyrate + 0.0544NH_3 + 0.0544CO2 + 1.7818H_2O \longrightarrow 0.0544C_5H_7O_2N + 1.8909Acetate + 1.8909H_2$
Methanogenesis	$0.2644CO_2 + H_2 + 0.0058NH_3 \longrightarrow 0.0058C_5H_7O_2N + 0.5171H_2O + 0.2355CH_4$
	$Acetate + 0.022NH_3 \longrightarrow 0.022C_5H_7O_2N + 0.945CH_4 + 0.945CO_2 + 0.066H_2O$

Table 6: Mass balanced equations of the Angelidaki model. It must be noted that Angelidaki coupled directly H_2 producing and H_2 consuming reactions (Interspecies Hydrogen transfer). Cf. section II-2 .

The second model proposed is more complete and is a compilation of the equations proposed by Hills, Angelidaki, and Gottschalk (cf. above and section II.1) and for simplicity it is further called HAG model.

The catabolic reactions presented in section II.1 have the advantage to be representative of the different possible metabolisms in anaerobic biodegradation, and then are probably present in the MELiSSA liquefying compartment using autochtonous strains. But it presents the disadvantage to be not coupled with biomass production. It was tried to thermodynamically couple the catabolic reactions with the biosynthesis reactions using relations established by Roels (1983) for anaerobic growth, but it failed at the present time.

The system is composed of 18 different equations including catabolic and anabolic reactions. They are listed in table 7a. The coupling yield between catabolic and anabolic reactions has

been taken from the different biomass yields reported in the literature (table 8) (most of them have been already mentioned before). The coupling factors between anabolism and catabolism are reported in the table 7b.

Reactions	Coupling factor value
Coupling between catabolic reactions of acidogenesis of carbohydrate and lipids and biomass growth from hexose	0.1 mol hexose / mol biomass
Coupling between catabolic reaction of amino acids degradation and biomass growth from acetate.	0.01 mol acetate / mol biomass
Coupling between acetogenic reaction of VFA and biomass growth from acetate	0.05 mol acetate / mol biomass

Table 7b: Coupling between anabolic and catabolic reactions for the HAG model

	Insomuluble Organic matter (composition)				Hexose					
	Proteins	Carbohydrates	Lipids	Pool AA	C6H12O6	Free NH3	H2O	Acetate	Propionate	Butyrate
Degradation biomass	2,7696	0,6278	1,0438			0,2540	-0,6197			
Hydrolysis proteins	-1			1			-0,2059			
Hydrolysis Carbohydrates		-1			0,1667		-0,1667			

	Insomuluble Organic matter (composition)				Hexose					
	Proteins	Carbohydrates	Lipids	Pool AA	C6H12O6	Free NH3	H2O	Acetate	Propionate	Butyrate
Acidogénésis pool AA				-1		0,2694	-0,5083	0,1520	0,0125	0,0151
Homofermentative + prop ferment.					-1		0,6667	0,6667	1,3333	
Butyrate fermentation					-1					1
acetate fermentation					-1			3		
Hydrolysis Lipids + Acidogenesis			-1				-0,875	0,5		
Growth from acetate (C2) substrate ***						-1	3	-2,5		
Growth from hexose (C6) substrate					-0,8333	-1	3			

	Insomuluble Organic matter (composition)				Hexose					
	Proteins	Carbohydrates	Lipids	Pool AA	C6H12O6	Free NH3	H2O	Acetate	Propionate	Butyrate
Propionate acetogenesis Equation							-2	1	-1	
Butyrate acetogenesis Equation							-2	2		-1
IsoButyrate acetogenesis Equation							-4	1		
Valerate acetogenesis Equation							-4	2		
Isovalerate acetogenesis Equation							-4	2		
Caproate acetogenesis Equation							-4	3		
Isocaproate acetogenesis Equation							-6	2		
Growth from acetate (C2) substrate ***						-1	3	-2,5		

	Insomuluble Organic matter (composition)				Hexose					
	Proteins	Carbohydrates	Lipids	Pool AA	C6H12O6	Free NH3	H2O	Acetate	Propionate	Butyrate
Acetoclastic equation**						-0,022	0,066	-1		
H2 methonogens**						-0,0109	0,978			

(Continued)

	Isobutyrate	Valerate	Isovalerate	Caproate	Isocaproate	CO2	CH4	H2	Biomass	H2SO4
Degradation biomass						0,558820628			-1	
Hydrolysis proteins										
Hydrolysis Carbohydrates										

	Isobutyrate	Valerate	Isovalerate	Caproate	Isocaproate	CO2	CH4	H2	Biomass	H2SO4
Acidogénésis pool AA	0,0076	0,0125	0,0177	0,0173	0,0099	0,2527		0,3406		0,0064
Homofermentative + prop ferment.						0,6667				
Butyrate fermentation						2		2		
acetate fermentation										
Hydrolysis Lipids + Acidogenesis								0,875		
Growth from acetate (C2) substrate ***									1	
Growth from hexose (C6) substrate									1	

	Isobutyrate	Valerate	Isovalerate	Caproate	Isocaproate	CO2	CH4	H2	Biomass	H2SO4
Propionate acetogenesis Equation						1		3		
Butyrate acetogenesis Equation								2		
IsoButyrate acetogenesis Equation	-1					2		6		
Valerate acetogenesis Equation		-1				1		5		
Isovalerate acetogenesis Equation			-1			1		5		
Caproate acetogenesis Equation				-1				4		
Isocaproate acetogenesis Equation					-1	2		8		
Growth from acetate (C2) substrate ***									1	

	Isobutyrate	Valerate	Isovalerate	Caproate	Isocaproate	CO2	CH4	H2	Biomass	H2SO4
Acetoclastic equation**						0,945	0,945		0,022	
H2 methonogens**						-0,4999	0,4452	-1,8909	0,0109	

Table 7a: Stoichiometric coefficients of the equations of the HAG model.
The growth equations (anabolism) are grouped with the group of catabolic reactions to which they are related.
***For the acidogens hydrolysing the amino acids, the growth is assumed from acetate substrate.
The equation is the same as the growth equation for acetogens

During the first tests of these models, it appeared that the biomass content of the substrate (cf. II.4.2.1) must be considered as a degradable organic matter by the autochtonous strains. This biodegradability is confirmed by the experiments with a pretreatment of the substrate by acidification or sonication, as with or without pretreatment (which degrade biomass into organic matter), the process efficiencies are unchanged. Then, in the Hills-Angelidaki model, the biomass was included in the definition of the degradable matter, and in the HAG model, a 19th equation was added in order to describe the hydrolysis of the biomass into proteins, carbohydrate, lipids, NH_3 and CO_2 (table 7a).

Process step	Yield	Reference
Acidogenesis	13.9375 g biomass/mol [$C_6H_{10}O_5$] 0.0929 Cmol biomass/Cmol [$C_6H_{10}O_5$]	Angelidaki et al., 1993
	0.03 Cmol biomass/ Cmol whey	Dunn et al, 1992
Acetogenesis	5.725 g biomass/mol propionate 0.0763 Cmol biomass/ Cmol propionate	Angelidaki et al., 1993
	0.04 Cmol biomass/ Cmol propionate	Dunn et al, 1992
	6.8 g biomass/ mol butyrate 0.068 Cmol biomass/Cmol butyrate	Angelidaki et al., 1993
	0.05 Cmol biomass/ Cmol butyrate	Dunn et al, 1992
Methanogenesis	0.75129 g biomass/ mole H2 0.0288 Cmol biomass/mole H2	Angelidaki et al., 1993
	0.026 Cmol biomass/ mole H2	Dunn et al, 1992
	2.8359 g biomass/mole CO2 0.1091 Cbiomass/mole CO2	Angelidaki et al., 1993
	2.75 g biomass/mol acetate 0.055 Cmol biomass/Cmol acetate	Angelidaki et al., 1993
	0.05 Cmol biomass/Cmol acetate	Dunn et al, 1992
Glucose → lactate	0.13-0.24 Cmol biomass/Cmol glucose	Roels, 1983
Acetic acid→methane	0.057 Cmol biomass/Cmol acetate	Roels, 1983
Glucose→2/3 propionate + 1/3 acetate + CO2	0.238 Cmol biomass/Cmol glucose	Roels, 1983
Glucose→methane+CO2	0.26 Cmol biomass/Cmol glucose	Roels, 1983

Table 8: Biomass yield coefficients .

II.4.2 - Comparison of the 2 mass balanced models

The two mass balance models have been represented in an Excel® spreadsheet. Some variable can be manipulated:

- the substrate composition
- the conversion factors (varying from 0 to 1)
- the biomass coupling factors, if they are required

II.4.2.1 - Inputs of the compartment: The faeces definition

Faeces, which are the principal substrate of the liquefying compartment are a complex substrate. Their composition are variable, then for modelling, only a mean composition is used (table 9). The composition used was obtained from the analyses reported in TN 26.1, TN 22.5 and TN 22.4. It can be outlined that the ash content (i.e. minerals) is not considered in the mass balanced models. Up to now, it is assumed that minerals directly flow through the reactor. In fact a part is used by the micro-organism biomass which contains minerals.

It is known that faeces contain fibres. At the present time, the fibre part was not analysed, and is defined as carbohydrate. As fibre are considered as non degradable matter (very low degradability), it is a mistake to assume that they are carbohydrate, which are considered to be degraded to hexose. At the present time, the problem of fibre is not fixed. The quantitfication, the composition and the biodegradbility of fibre contained in faeces stay to be determined..

Assuming an elemental composition for proteins, lipids and carbohydrates, the CHONSP formula of faeces can be calculated (table 9).

As can be seen in table 9, one third of the faecal organic matter is assumed to be composed of biomass (TN 22.4). In their mass balance calculation, Demey et al. assumed that this biomass was not degraded and then they corrected the raw biodegradation efficiency with the biomass content (table 3). But it is evident that a large amount of this biomass is dead in the thermophilic process of anaerobic biodegradation.
The experiments concerning the pretreatment of faecal material by acidification and sonication have shown that the efficiency was not enhanced by the treatment which have broken the biomass. From these experiments it seems then that the biomass content of faeces is degraded and has to be included in the definition of the organic matter to degrade. It could be then wrong to define an efficiency for the degradation of organic matter without biomass. Of course this remark can only be applied for the dead biomass and not for the active (alive) one.

The mean human faeces description reported in table 9, as wet, dry, organic matter and VFA is thus subject to modification (mainly when a fibre content will be available).

II.4.2.2 - Comparison of the 2 proposed models.

For each of the models, a "conversion factor" must be associated to each stoichiometry. For the Hills-Angelidaki-Gottschalk (HAG) model we have developed, a coupling factor between catabolic and anabolic reactions must be given too.

	Wet % mass	Dry Matter % mass	Organic Matter %mass	Organic Matter %mass	VFA %mass
Water	71				
Dry matter	29	100			
Om		86	100	100	
Ash		14			
Solides Suspensions					
Volatil suspended solid					
COD tot		127			
COD Sol					
TOC					
NH4+		0,5			
NO3-		0			
N tot		5,1			
N orga		4,6	5,35	5,35	
Proteiens		30	34,88	10,09	
Carbohydrates			50,00	45,73	
Lipids			10,47	6,20	
VFA		4	4,65	4,65	
Biomass				33,33	
Acetate		2	2,33	2,33	50,00
proplonate		0,64	0,74	0,74	16,00
Iso-butyrate		0,1	0,12	0,12	2,50
butyrate		0,732	0,85	0,85	18,30
Isovalerate		0,092	0,11	0,11	2,30
Valeatre		0,196	0,23	0,23	4,90
Isocaproate		0,028	0,03	0,03	0,70
Capoate		0,168	0,20	0,20	4,20

Mass	Composition					
(g)	C	H	O	N	S	P
18						
22,64	1,0000	1,5683	0,3063	0,2694	0,0064	
27,00	1,0000	1,6667	0,8333			
16,00	1,0000	2,0000	0,1250			
22,60	1,0000	1,4000	0,4000	0,2000		
60,00						
74,00						
88,00						
88,00						
102,00						
102,00						
116,00						
116,00						

Table 9: Mean human faeces detailed composition

The two models can be compared together by observing the respective efficiencies that they give as result for the modelling of the same process. A 'same anaerobic process' will be defined by using:

- the same substrate
- the same setting values for the conversion factor associated to the different reactions.

Because there is not the same number of reactions in the 2 models, a correspondence between the reactions, and their conversion factors is assumed (table 10).

Step Conversion factor (SC)	Model Hills-Angelidaki	Model HAG
SC1 Hydrolysis step	Insoluble matter hydrolysis factor Proteins (NH3 content) hydrolysis factor	Carbohydrate hydrolysis Proteins hydrolysis Lipids hydrolysis (+acidogenesis)
SC2 Acidogenic step	Acidogenesis conversion factor of soluble matter	Acidogenesis of amino acids Acidogenesis of hexose to acetate Acidogenesis of hexose to butyrate Acidogenesis of hexose to propionate
SC3 Acetogenesis step	Butyrate acetogenesis conversion factor Propionate acetogenesis conversion factor	Conversion factors for 7 VFA to acetate
SC4 Hydrogenomethanogens	Conversion factor of H_2 to CH_4	Conversion factor of H_2 to CH_4
SC5 Acetomethanogens	Conversion of acetate to CH_4	Conversion of acetate to CH_4

Table 10: Correspondence of the conversions factors for the two proposed mass balanced models. Report to table 7a , for the association of conversion factors with equations.

The comparison was made on the basis of the variation of the conversion factors affected to the hydrolysis step (SC1) for the two processes (table 11). Two kind of processes were compared:

- a complete anaerobic process, up to methanogenesis
- an inhibited process for methane production (i.e. SC4 and SC5 factors are set to 0).

The setting values of the conversion factor, used for the comparison of the two models are reported in table 11. In the related figures , only SC1 values are used as abscissa.

	SC1	SC2	SC3	SC4	SC5
Complete process	from 0.2 to 1	1	1	1	1
Methanogenesis inhibited	from 0.2 to 1	1	1	0	0

Table 11: Value of the conversion factor for the comparison of the 2 models.

The models are compared from the calculation of their respective efficiencies (biogas efficiency, VFA efficiency), from the gas composition at the end of the process, the N-balance and the biomass growth associated to the process. The results are presented in figures 2 and 3.

Biogas means methane, hydrogen and carbon dioxide production. It must be kept in mind that the gas production of ammonia or VFA was not taken into account, and that the dissolved forms of carbon dioxide (in liquid at pH 8, there is 1 CO_2 for 45 C total) are not calculated.

II.4.2.3 - Discussions - Conclusions

The 2 mass balance models are available in an EXCEL® spreadsheet format.

For the complete anaerobic biodegradation process, it can be noticed that, for the 2 models, the maximum biodegradation efficiency (given by the biogas production efficiency), can not be greater than 85%. Considering the total biodegradation efficiency calculated from biogas production in the different experiments (table 3), if it is assumed that all steps of the process (excepting hydrolysis) are complete (conversion factor equal to 1), the hydrolysis step is then the limiting step, with a mean efficiency around 50%.

The biogas efficiency in an inhibited process can not be greater than 35 %. This indicates that the use of such a criteria to determine the efficiency of this process is not accurate. The VFA efficiency (or other C-products efficiencies) is a better criteria.

For the complete anaerobic process, the two models are roughly identical on the point of view of biogas efficiency. Nevertheless the HAG model presents a more variable biogas composition than the Hills-Angelidaki model. The most important difference between the 2 models concerns the N-balance and the biomass production. For the Hills-Angelidaki model, the biomass produced is 0.1 g/g OM degraded and for the HAG model, this biomass is 0.25 g/g OM degraded. This difference gives for the HAG model a N-organic/N-total ratio greater than that of the Hills-Angelidaki model. This difference comes from the assumptions for the coupling between anabolic and catabolic reactions in the HAG model. The ratio N-organic/N-total seems then to be a useful tool to determine if these coupling factors have been badly estimated

For the inhibited process, the same remarks can be made.

Hills-Angelidaki model:

Advantages: it is simpler (5 equations) than the HAG model. It was validated with experiments of cattle manure biodegradation.

Disadvantages: It is difficult to adapt the substrate representation of the model to the definition (proteins, carbohydrate, lipids) of the human faeces. It is not known if the equations proposed can be used for human faeces degradation.

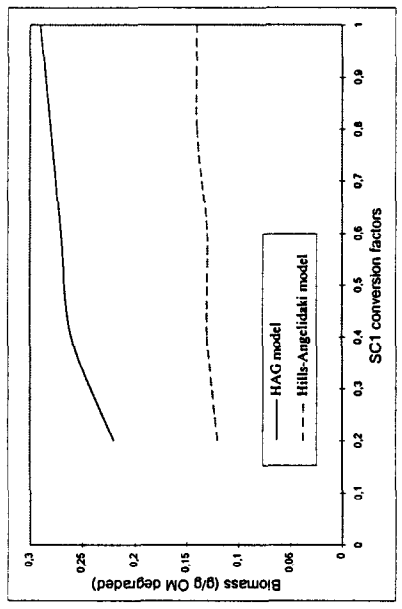
HAG model:

Advantages: With more reactions, it is attempted that the model can be adapted to more different situations. The hydrolysis step has been adapted to the human faeces composition definition.

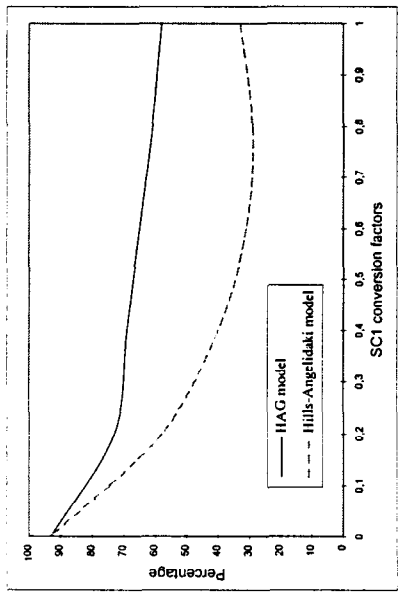
Disadvantages: it requires coupling between anabolism (biomass) and catabolism. It contains a lot of equations (19 equations), and increases then the number of parameters to know (coupling between anabolism and catabolism, kinetic parameters).

It is difficult to choose between the 2 models at the present time. They must be compared in dynamic simulations to determine if the increase of equations and parameters of the HAG model has a real interest. It could be of interest to modify the description of the hydrolysis step in the Hills-Angelidaki model by using the one of the HAG model.

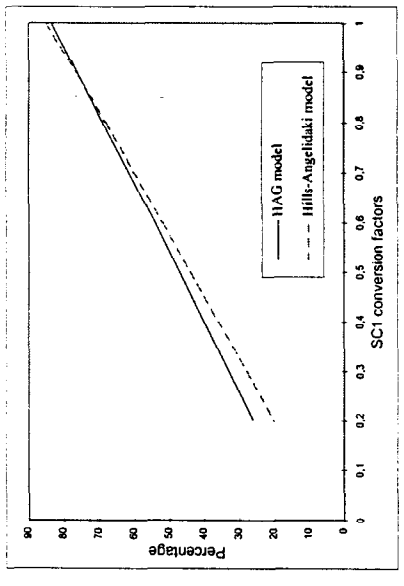
The mass balance model can be representative only of a steady state process (in continuous conditions). The mass balance model can be used calculate the efficiency of the process only by assuming a steady state (i.e. the conversion factors of each equation are stables). In experiments TI and TI+CL, by feeding at regular interval the reactor, a pseudo continuous process was obtained and it seems that a steady state was reached after 50 hours of processing. In such situations, I think that the use of a mass balance model on a feeding period can be justified.



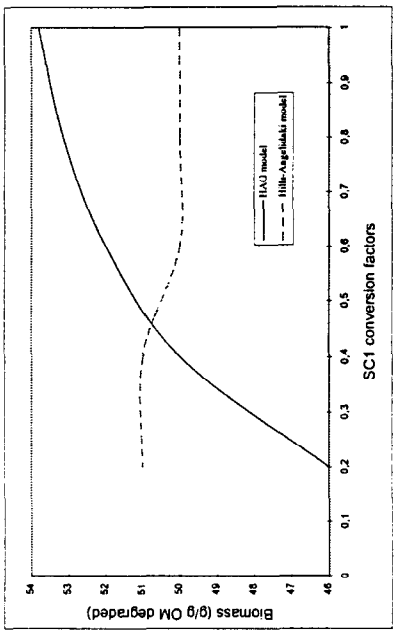
Biomass production



N-organic/N-total ratio

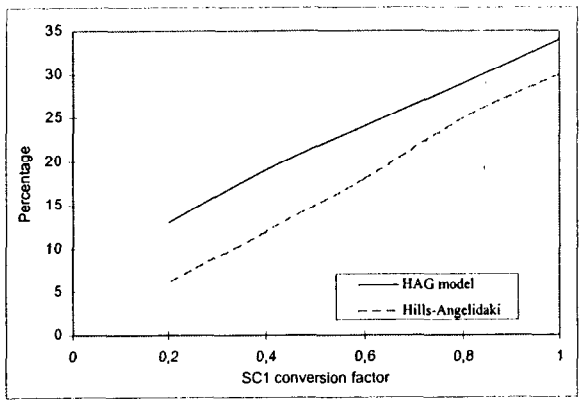


Biogas efficiency

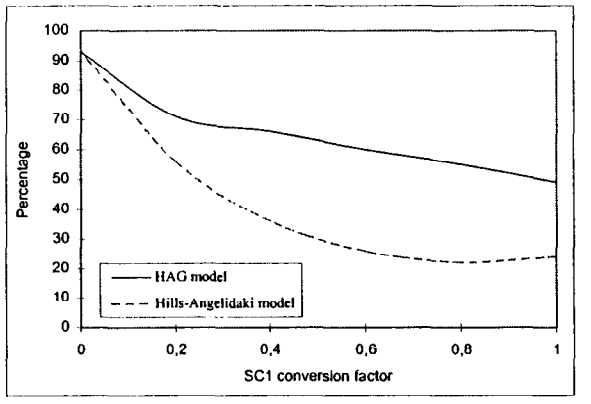


Biogas methane composition

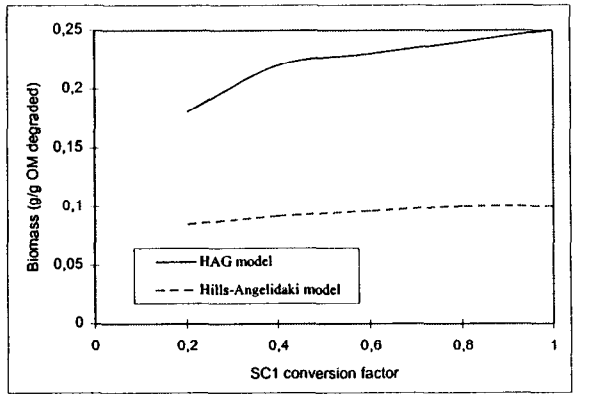
Figures 2 : Comparison of the HAG and the Hills-Angelidaki models for a complete anaerobic biodegradation process



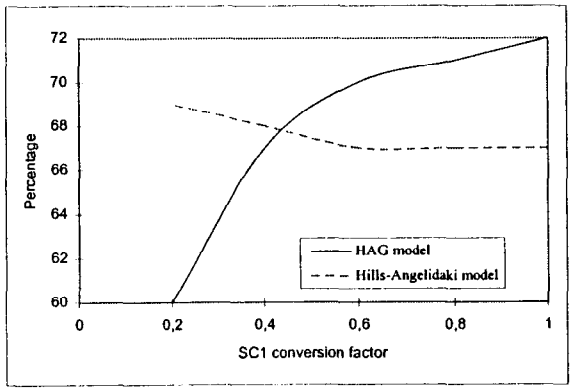
Biogas efficiency



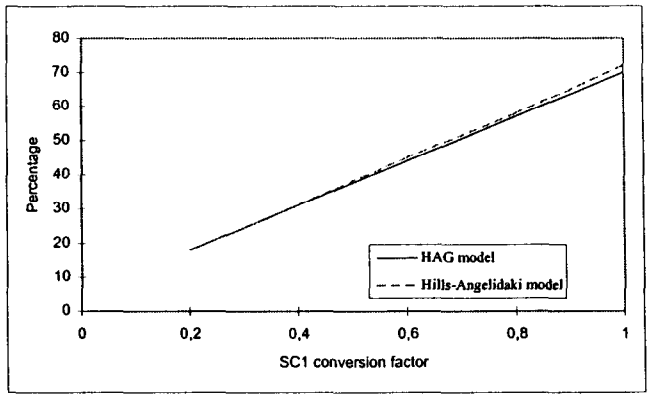
N-organic/N-total ratio



Biomass production



Biogas hydrogen composition



VFA efficiency

Figures 3 : Comparison of the HAG and the Hills-Angelidaki models for an anaerobic biodegradation process in which methanogenesis is completely inhibited

II.4.3 - Bases for dynamic simulation of batch (or continuous) experiments.
In this chapter, the objective is not yet to present a fully complete and functional dynamic model of the anaerobic process for the compartment I, but to expose the bases of this model.

The steps for the building of the dynamic model are:

1- the precise description of the process design (including feeding and recycling regimes)
2- the choice of a biological mass balance model
3- the choice of the biological kinetics and of the kinetic parameters (maximum growth rates, decay rates, saturation and inhibitory constants).
4- The development of the physical model (gas/liquid equilibria, pH equilibria, temperature effect).

II.4.3.1 - Process design
The fed-batch reactors in EPAS were run in order to observe and to study the efficiency of the degradation of human faeces and cellulose under methanogenesis inhibited or not inhibited conditions.
To avoid methane production in the MELiSSA loop, the methanogenesis must be inhibited. Experiments have shown that in batch processes where gas is flowing out of the reactor only by over pressure, the inhibition of methanogenesis reduce the degradation efficiency of the process. It is known that a partial hydrogen pressure up to 10^{-4} atm has an inhibitory effect on the acetogens. The use of a continuous gas flow (inert gas free of H_2 and oxygen) through the reactor would reduce this effect.
An overview of a possible design for the reactor is given in figure 6. If pretreatments or recycling are used, their effects on the quality of the substrate must be known (as the separation efficiency and pre-acidification that have been detailed in TN 26.2 and TN 32.2).
Another important point is to clearly define the feeding regime, as well as the recycling regime, and to understand how the feeding material is defined (organic matter, wet faeces...; the definitions must be based on a table giving the composition of the matter, as in table 9).

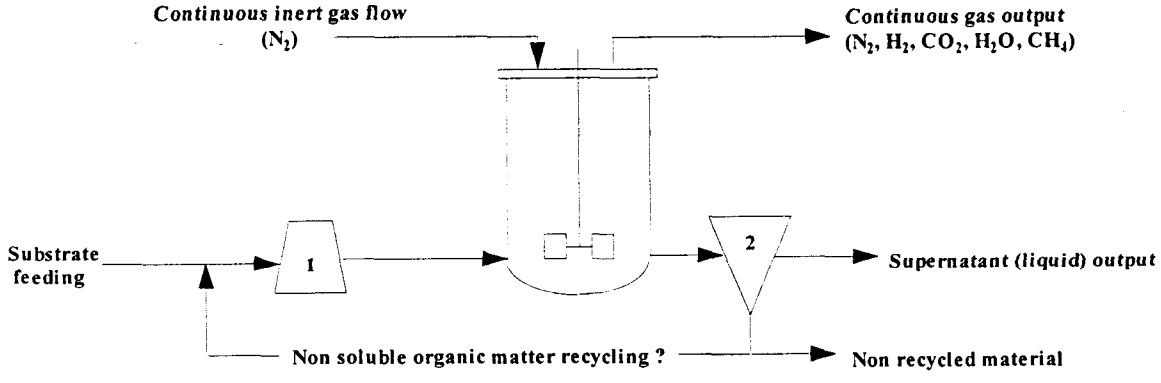


Figure 6: Proposed design for the reactor. 1 is a pretreatment process (or post-treatment if there is recycling). 2 is the separation process for recycling and/or liquid output of the reactor. It is evident that at least an efficiency of these processes must be know for their integration in a dynamic model.

In order to perform simulations of the process and to compare them with experiments it is important to know:

- the initial conditions of the reactor (volume, medium, and if possible inoculum)
- the substrate composition
- the products formed (qualitatively and quantitatively)
- the hydrodynamics (feeding regime, gas flow rate)
- the physical conditions (pH, temperature)

II.4.3.2 - Bases for a biological dynamic model

The 2 mass balance (stoichiometries) models previously presented can be used as the biological models for dynamic modelling. It would be interesting to compare dynamic simulations with the 2 models.

A survey of different kinetic models has been reported in TN 27.1. The most simple kinetic model that can be used is a Monod based model at which can be added an inhibitory and a decay rate term. This is such a model which was used by Angelidaki and Dunn for dynamic modelling(cf. II.2).

For each equation, the growth rate (r_X) and the substrate consumption/product formation rates (r_{Si}) are expressed as:

$$\begin{cases} r_X = \mu . X - k_D . X \\ r_{Si} = \dfrac{-1}{Y_{X/Si}} \mu X \end{cases}$$

$$K_{I-k} = 1 + \frac{C_k}{I_k}$$

$$\mu = \mu_{max} \prod_k \frac{C_k}{(Ks_k + C_k)} \frac{1}{K_{I-k}}$$

where: C_i is the concentration of compound i

I_i is the inhibitory constant of compound i.

μ_{max} is the maximum specific growth rate

$Y_{X/Si}$ is the biomass growth yield for the substrate Si

The kinetic parameters used by Angelidaki and Dunn are reported in table 12. A great difference between the kinetic parameters of the two models can be noted. The same remark can be made on the kinetic parameters reported by Demey in TN 26.1. These variations can result from the fact that we consider different strains in different processes. This is the major problem when using unknown consortium of bacteria in a process.

Step	μ_{max}	Remarks and references	Saturation (Ks) constants	References	Inhibitory (Ki) constants	References
Hydrolysis	1 d^{-1} 0.042 h^{-1}	Concentrations in g/l Substrate $C_5H_{10}O_5nNH_3$ Angelidaki et al 1993			0.33 g/l (VFA)	Substrate $C_5H_{10}O_5nNH_3$ Angelidaki et al 1993
Acidogenesis	5 d^{-1} 0.208 h^{-1}	Concentrations in g/l Substrate $C_5H_{10}O_5$ Angelidaki et al 1993	0.5 g/l 0.0033 mol/l	Substrate $C_5H_{10}O_5$ Angelidaki et al 1993		
Hydrolysis + acidogenesis	0.4 h^{-1}	Concentrations in Cmol/m3 Substrate Whey Dunn et al 1992	0.25 Cmol/m3	Substrate Whey Dunn et al 1992		
Propionate degradation	0.54 d^{-1} 0.0225 h^{-1} 0.011 h^{-1}	Concentrations in g/l Coupled with H_2 consumption Angelidaki et al 1993 Concentrations in Cmol/m3 Dunn et al. 1992	0.259 g/l 0.0035 mol/l 0.0074 Cmol/m3 2.46 10^{-6}mol/l	Coupled with H_2 consumption Angelidaki et al 1993 Dunn et al. 1992	0.96 g/l (acetate) 0.0057 mol/l	Angelidaki et al 1993
Butyrate degradation	0.68 d^{-1} 0.0283 h^{-1} 0.005 h^{-1}	Concentrations in g/l Coupled with H_2 consumption Angelidaki et al 1993 Concentrations in Cmol/m3 Dunn et al. 1992	0.176 g/l 0.002 mol/l 0.032 Cmol/m3 8 10^{-6}mol/l	Coupled with H_2 consumption Angelidaki et al 1993 Dunn et al. 1992	0.72 g/l (acetate) 0.012 mol/l	Angelidaki et al 1993
Aceticlastic step	0.6 d^{-1} 0.025 h^{-1} 0.008 h^{-1}	Concentrations in g/l Angelidaki et al 1993 Concentrations in Cmol/m3 Dunn et al. 1992	0.120 g/l 0.002 mol/l 0.1 cmol/m3 5 10^{-5} mol/l	Angelidaki et al 1993 Dunn et al. 1992	0.26 g/l (NH3) 0.0153 mol/l 1.4 cmol/m3 (?NH3) 1.4 10^{-3} mol/l	 Dunn et al. 1992
H2 methantogens	0.058 h^{-1}	Concentrations in Cmol/m3 Dunn et al. 1992	0.001 mol/m3 10^{-6} mol/l	Dunn et al. 1992		
Decay rate	5% of μ_{max} 0.0004 h^{-1}	Angelidaki et al 1993 Dunn et al. 1992				
Inoculum (From Dunn et al. 1992)	30.0 41.0 100.0 18.2 2.0	Acidogens Cmol/m3 Butyrate acetogens Cmol/m3 Aceticlastic meth. Cmol/m3 Propionate acetogens Cmol/m3 H2 methanogens Cmol/m3				

Table 12: Kinetic parameters of the dynamic model of Angelidaki and Dunn.

For the modelling of the physical reactions involved in the process (pH equilibria, gas/liquid transfer), the chemical equilibria and transfer laws that will be used have been already detailed in TN 17.1, TN 23.1 and TN 27.1.

II.4.3.3- First dynamic simulations.

A dynamic model was build to try to simulate the batch processes operated the past two years by EPAS (cf. chapter I). This first model is based upon the HAG model. The gas liquid equilibria is taken into account, and it is assumed that there is a perfect equilibria. The effect of pH is taken into account in the gas/liquid equilibria by using the partition coefficients calculated in TN 17.1 and 23.1.
The gas flow rate is calculated by assuming that the pressure inside the reactor is maintained at 1 atm (i.e. the volume of gas produced is the gas flow rate).

The kinetic parameters where taken from table 12.

It was tried to simulate some of the experiments conducted by EPAS.
Because each experiment was initiated from the content of one previous reactor, it was first tried to simulate the experiment called process run I (table 2), which can be considered as the star-up reactor for all the other experiments. The results of the simulation we have obtained were completely different of the experimental ones, and it can be concluded that the simulation of this reactor has failed. It must be outlined that the process run I was operated for the degradation of non diluted faeces, what gives difficult the definition of a liquid phase and of the concentrations that are involved in the model.

Nevertheless, it was tried to simulate other experiments where faeces are diluted. The 2 processes simulated were the TI reactor (or TI+CL, the difference between the two experiments is only the inoculum), and the Trc/Trcf reactor [the results of these 2 experiments are presented in TN 34.2 and some of them are reported in the appendix of this TN]. The initial content of the reactor for the compounds measured (VFA, NH_3) was fixed to the measured values of these compounds at the time t=0 of the experiment.

The major problem encountered was the initialisation of the different biomass groups (acidogens, acetogens, methanogens).
For the TI process this initialisation was made in order to have an evolution of the VFA compatible with the experimental measurements.
For the Trc/Trcf process, the biomass initialisation was made using the concentration values of the different biomass obtained at the end of the simulation of the process TI. This can be made because the content of the TI reactor is used to start-up the Trc/Trcf reactor (table2).

The results of these first simulations are reported in figures 4 (TI reactor) and 5 (Trc/Trcf reactor). The simulation curves must be compared with the experimental ones presented in TN 34.2 and reported in appendix. From this comparison, it can be concluded that:

1 - The biomass initialisation is not a trivial problem. Very different situations can be obtained for different ratio of the group of bacteria responsible of the different steps of the process. Its seems that the production of high amount of acetate is the result of an

insufficient quantity of acetoclastic methanogens. It can be noted that this population represent 50% of the total biomass in the initial conditions defined by Dunn (table 12).

2 - For the TI (or TI+Cl) reactor simulation (figures 4). The profile for the VFA production is intermediate between those of the two experiments TI and TI+Cl. In experiment TI, the decrease of VFA is observed at day 50 (figure 3.5 reported in appendix). In simulation, the decrease is much slower and starts at day 25. This suggests an inadequation between the kinetic parameters used and the process. The gas production is roughly the same in the simulation and in experiments, but the gas composition is not stable. The often observed 60% of methane ratio is reached but it falls at the end to 48%. No Hydrogen is produced, and it is like if the CO_2 production increases. The methane production reaches a steady state, while CO_2 continues to increase in the gas phase. It is not known if it is problem of initialisation of the process (the liquid phase is at the beginning assumed to be free of CO_2 and of carbonates) or if it is a problem with the gas liquid model chosen or if it is a numerical problem. It must be outlined that for various different biomass initial conditions, the biomass ratio obtained at the end of the simulation is roughly the same.

3 - For the Trc/Trcf reactor simulation (figures 5). It is hard to find a correspondence between simulations and experiment (figures 3.9, 3.10 and 3.11 reported in appendix), what means that a lot of work remains to be done for the building of a dynamic model. It can be noticed that after a feeding, the VFA production increases then decreases quickly. After feeding with faecal material (day 42) acetate production is greatly increased. This increase is not observed in experiment. Moreover, propionate became the major VFA during experiments, but never in the simulation. It is probably due to a bad assumption for the kinetic parameters. Considering that the biogas production is lower in simulation than in experiments, the problem is probably due to the kinetic parameters value for acetoclastic methanogens. Another point must be outlined: the methane ratio is very low in the simulation (figure 5c). At the start up, the dissolved carbon dioxide concentration obtained at the end of the TI simulation has been used, then the liquid phase is quickly in equilibria with the gas phase. It will be necessary in a further study of the dynamic model to determine if the problem of the gas ratio is a numerical problem or a biological model problem.

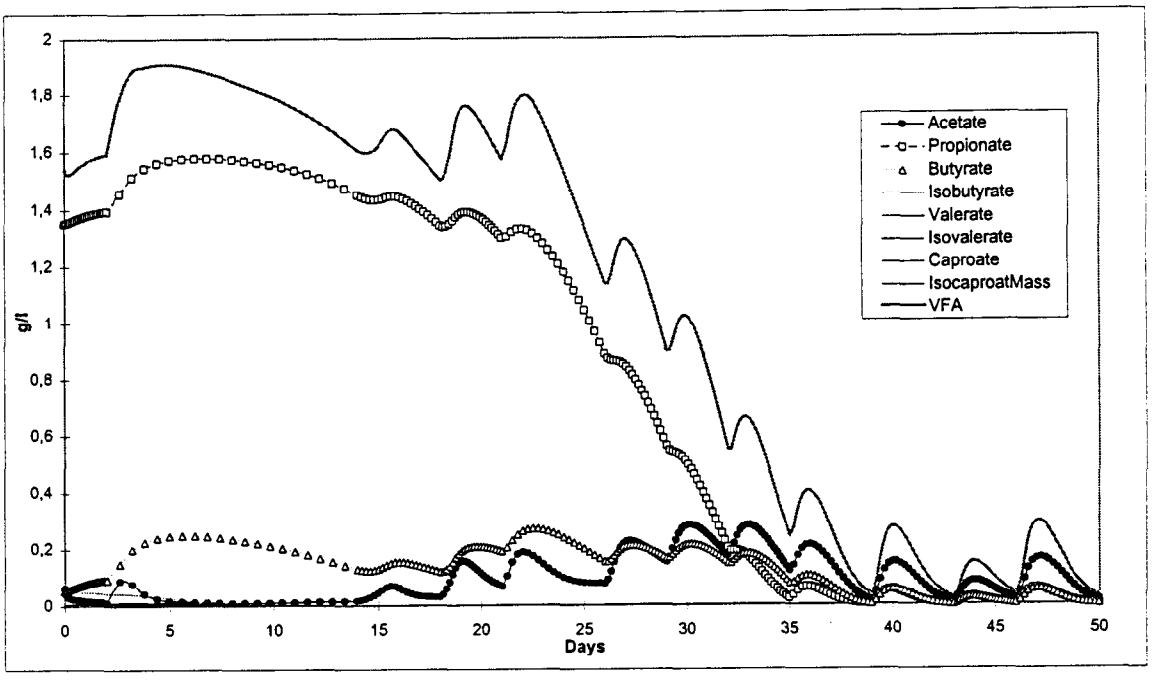


Figure 4a: Simulation of VFA production for reactor TI.
Oscillations are the result of the feeding regime
Curves must be compared with figure 3.5 of TN 34.2 and reported in appendix

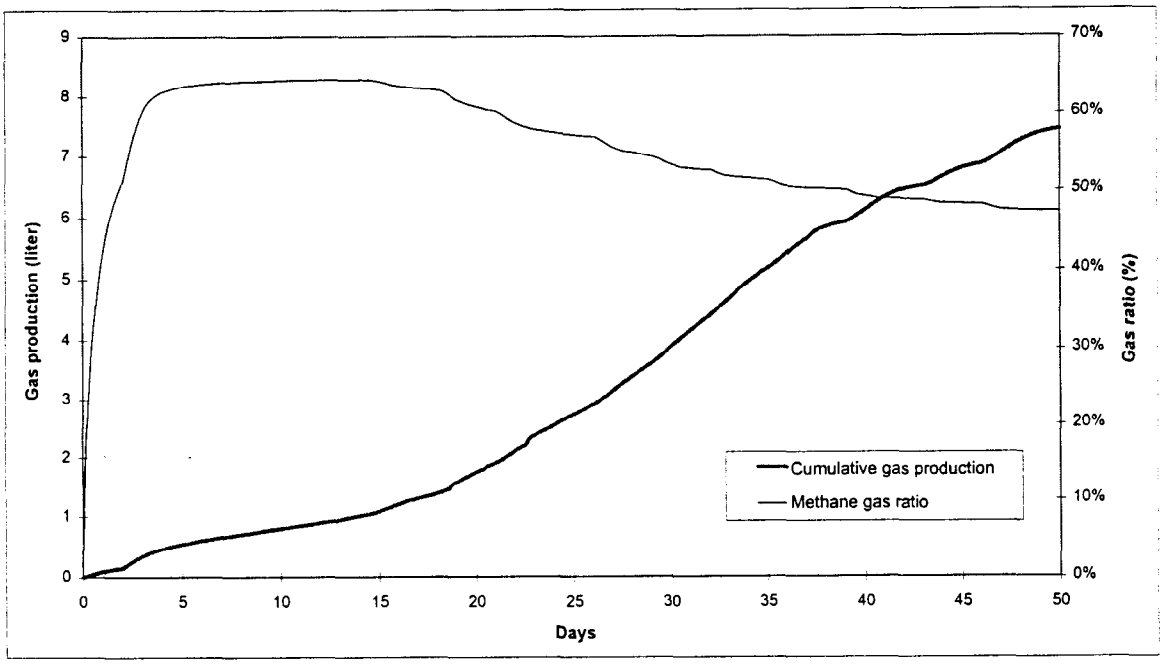


Figure 4b: Simulation of gas production and gas compostion for reactor TI
Oscillations are the result of the feeding regime
Curves must be compared with figure 3.4 of TN 34.2 and reported in appendix

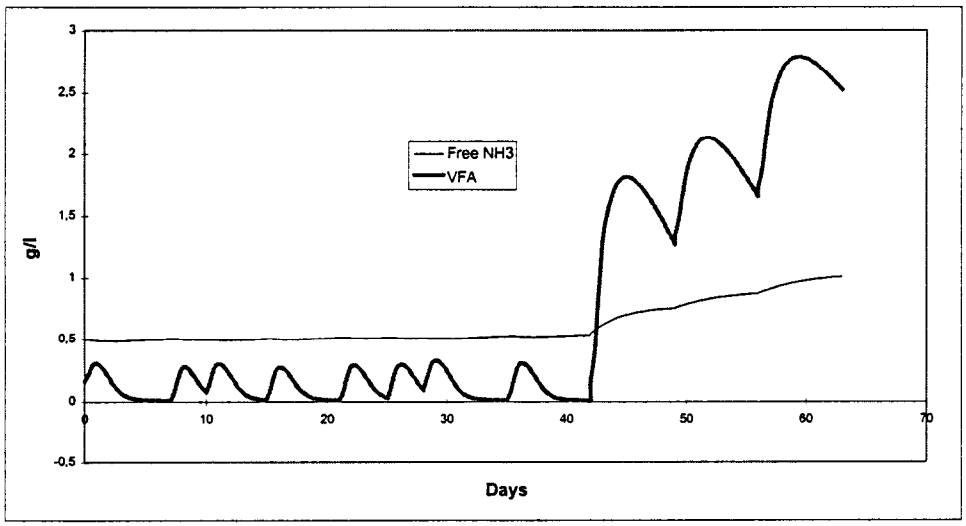


Figure 5a: VFA production and NH3 evolution for simulation of Trc/Trcf process
The curves must be compared with the figure 3.9 of TN 34.2 and reported in appendix

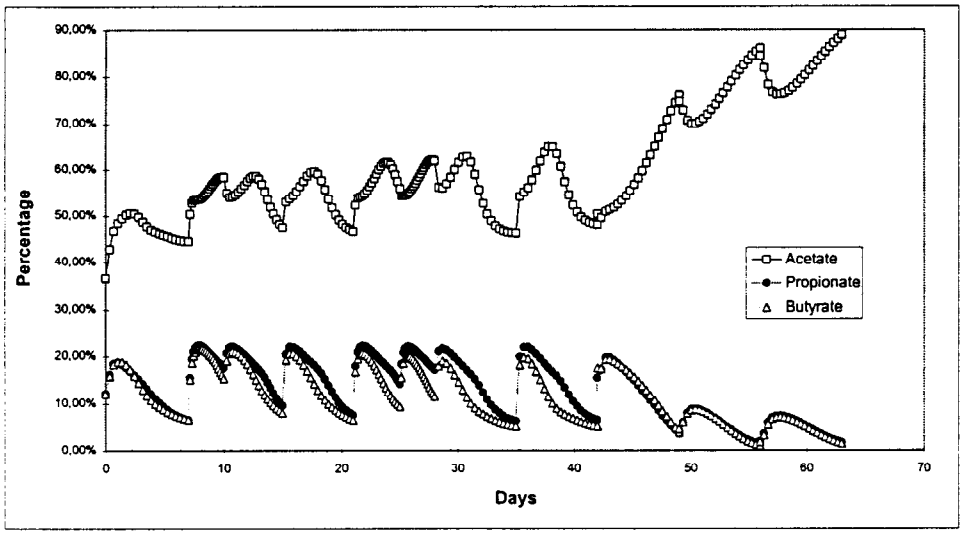


Figure 5b: VFA composition for Trc/trcf process simulation
The curves must be compared with the figure 3.10 of TN 34.2 and reported in appendix

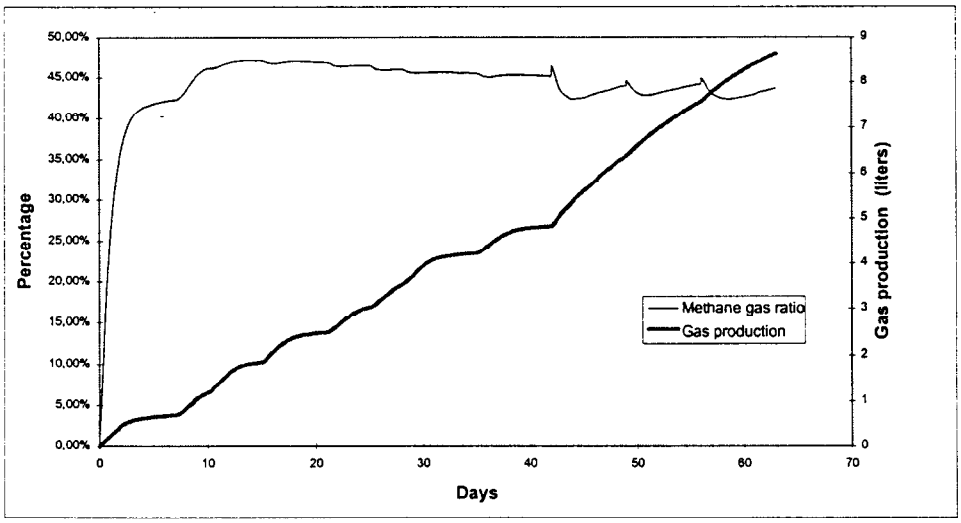


Figure 5c: Gas production and gas composition for Trc/Trcf process simulation
The curves must be compared with the figure 3.10 of TN 34.2 and reported in appendix

Conclusion

A review of the past experiments on the liquefying compartment and of the anaerobic reactions was made. This helps to summarise the current knowledge of the compartment.

Two mass balance model are proposed (stoichiometric models). Both have advantages and disadvantages. A comparison between the two models showed that the main differences concern the biomass production and the gas composition.

A preliminary dynamic model was build in order to identify the possible future problems for dynamic modelling. The stoichiometric model used was the HAG model. By using the literature kinetic parameters, it was difficult to simulate some of the past batch reactor experiments. The mains point to consider for the building of a future dynamic model would be:

- the precise definition of start up conditions, especially the ratio of the different group of bacteria carrying out the steps of anaerobic degradation (it is possible to estimate such conditions from a steady state simulation).
- the reactor design parameters (volumes, flow rates, temperature, pH), feeding regimes, and recycling regimes must well-defined.
-experiments with a continuous gas-flow rate are easier to model than experiments with an over-pressure system.

First simulations shown the difficulty to fit model and past experiments. The HAG model used presents probably some defaults (as the coupling factors assumed between anabolism and catabolism), but it seems too that the kinetic parameters used are not adapted to the process. For the future, it will be of interest to compare the two mass balance models in dynamic simulations. This would help choosing between the two models for the description of the compartment.

The present inaccuracy of the kinetic parameters suggests the need of the identification of these parameters from experiments led at EPAS. New experiments will be required where a maximum of the measurable data would be the following:

- VFA production and composition
- Gas composition (a continuous gas flow rate of N_2 would be used instead of over pressure, in order to keep a low hydrogen partial pressure)
- Other carbon products (such as lactate which could appear in inhibited processes)
- pH, temperature
- feeding regimes

An other important point to fix is the mean substrate composition (in term of wet matter, dry matter, organic matter, VFA, biomass) in order to have common definitions of substrate both for experiments and simulations.

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APPENDIX

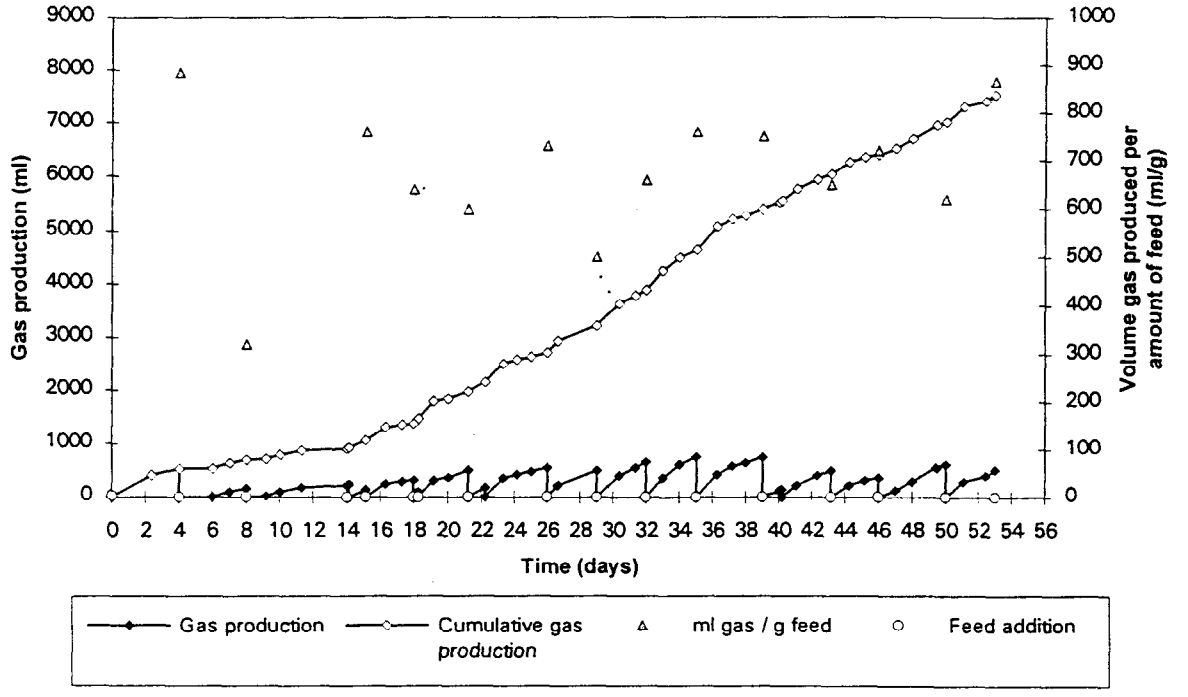

Figure 3-4. Feeding regime, cumulative biogasproduction and biogas production per amount of feed of the set-up with thermophilic inoculum (reactor "TI")

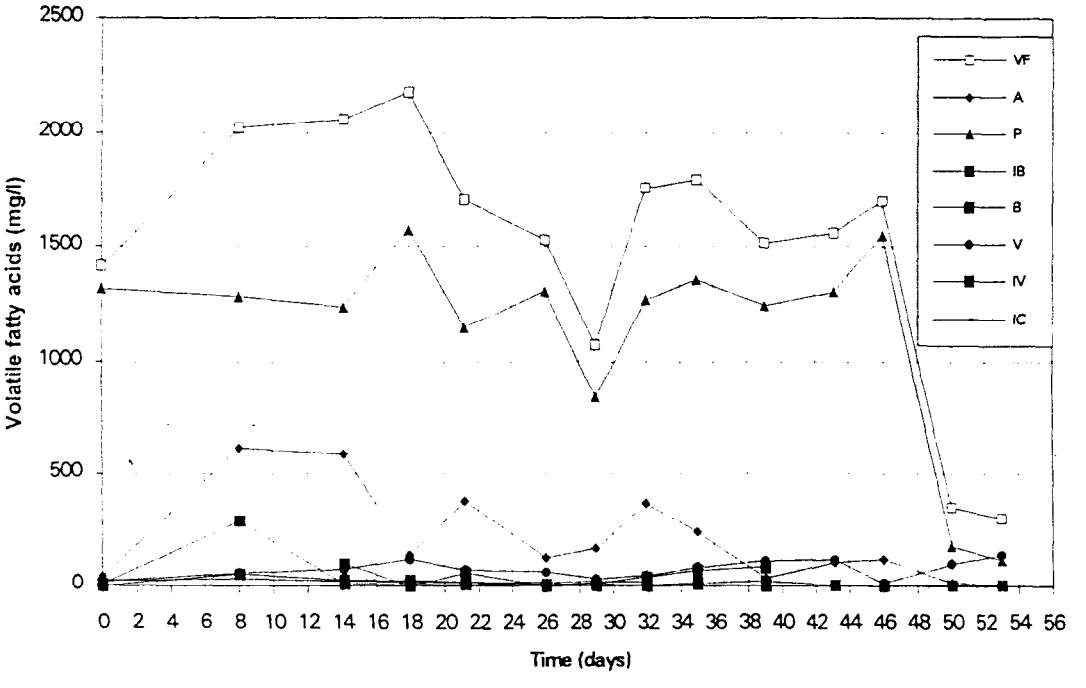

Figure 3-5. Evolution of the volatile fatty acid concentration in the set-up with thermophilic inoculum (reactor "TI")

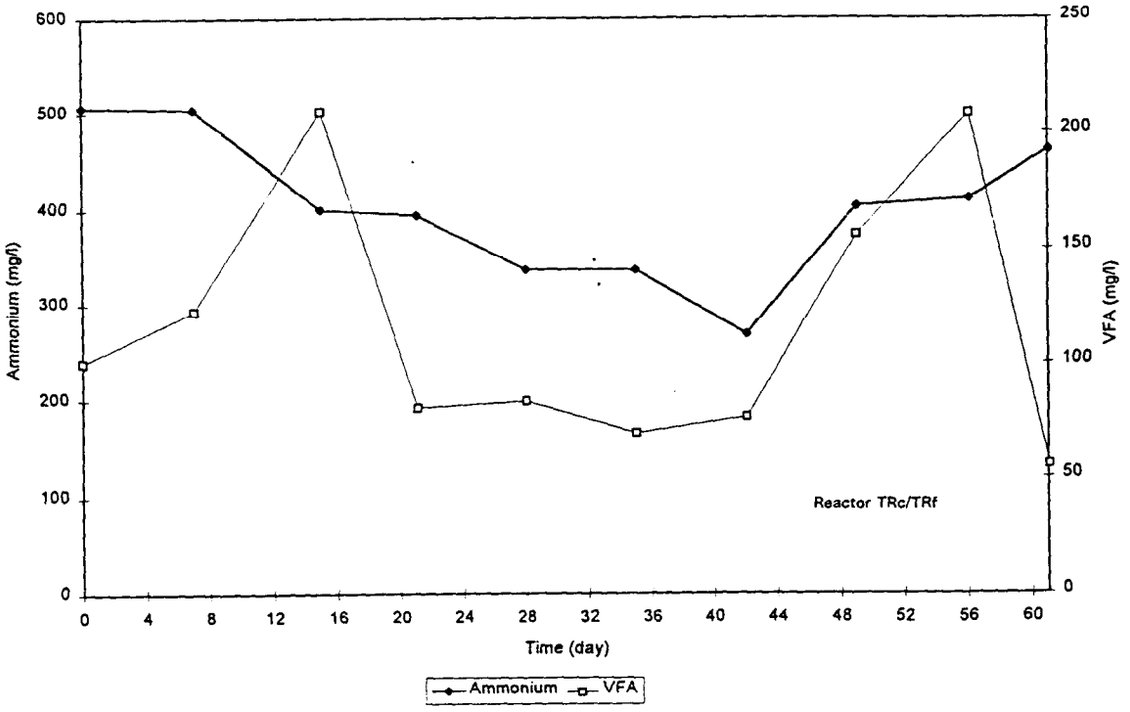

Figure 3-9. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRc/TRcf

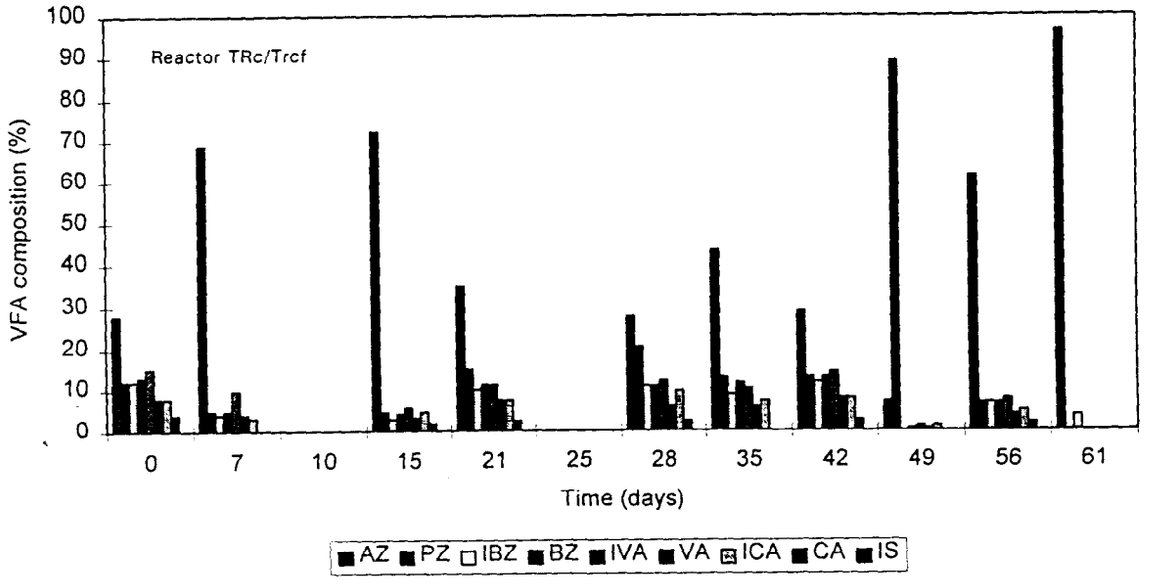

Figure 3-10. Composition of the volatile fatty acids produced in reactor TRc/TRcf

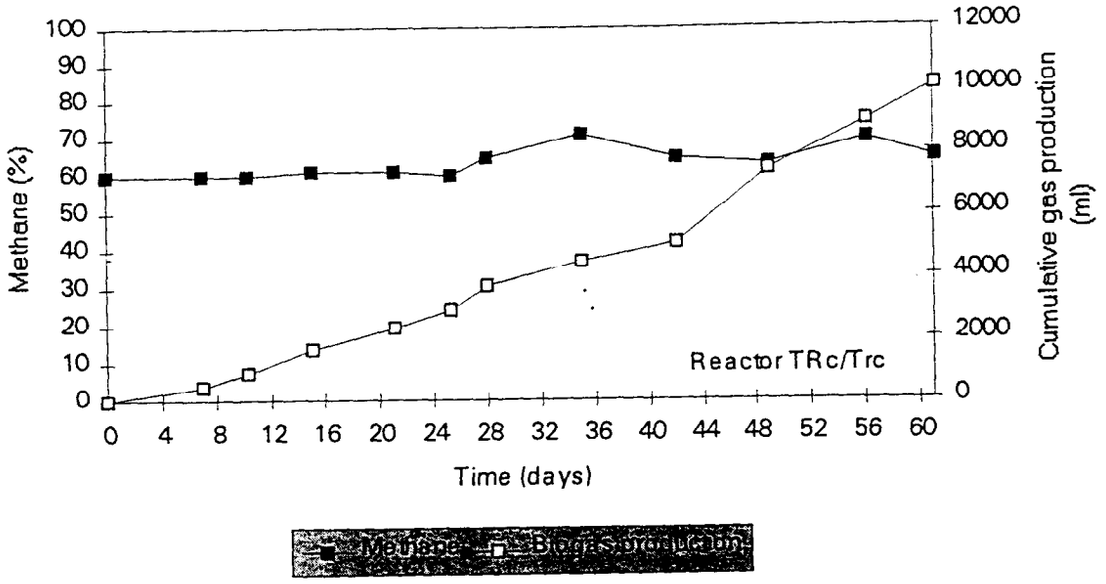

Figure 3-11. Evolution of the ~~ammonium concentration~~ and ~~volatile fatty acid concentration~~ in the reactor TRc/TRcf

methane cumulative gas production

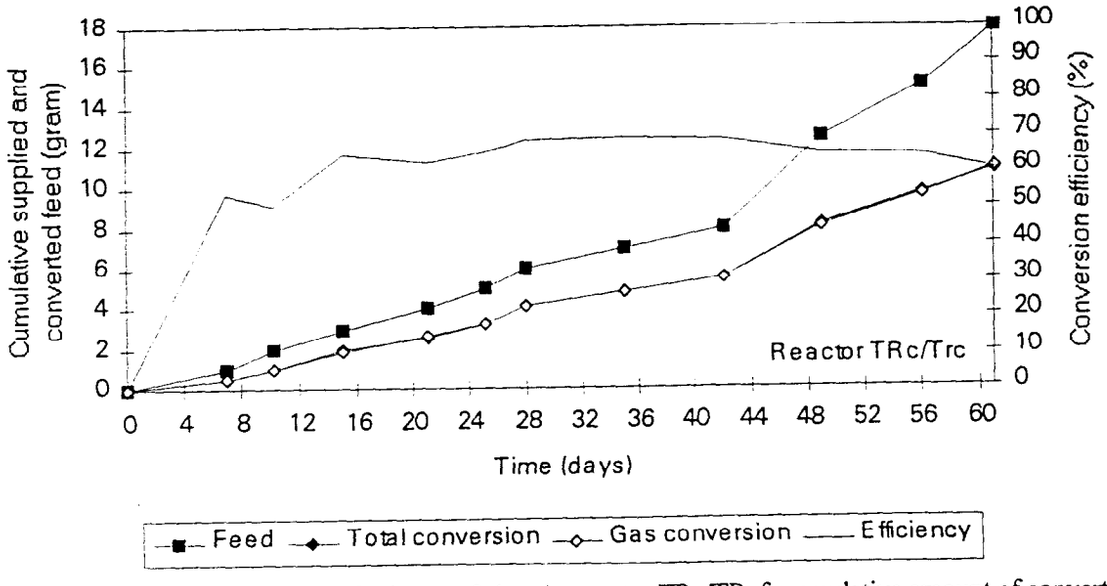

Figure 3-12. Cumulative amount of substrate fed to the reactor TRc/TRcf, cumulative amount of converted substrate and the calculated conversion efficiency