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Presentation of the Work Plan '95-'96

Stochiometric modelling of anaerobic biodegradation

Literature review of pre-treatment methods

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1. INTRODUCTION

It was shown in previous experiments (TN22.4) that that axenic cultures and mixed cultures of the selected strains *Clostridium thermocellum*, *Clostridium thermosaccharolyticum* and *Coprothermobacter proteolyticus* I8 were not able to biodegrade faecal material with a high efficiency. To improve the biodegradation of human faeces, an inoculum of autochtonous strains present in human faeces was cultivated (TN26.1 & TN26.2). This inoculum had proteolytic and cellulolytic capacities. In thermophilic conditions (55 °C), about 60 % of the total amount of proteins present in the human faeces were biodegraded and 40 % of the total amount of carbohydrates. Taking into account that about one third of the faeces consists of bacterial biomass, it can be calculated that about 90 % of the non-bacterial proteins present in the human faeces were biodegraded and 60 % of the non-bacterial carbohydrates. The fibrous fraction of the human faeces seems to be the most resistant part to biodegrade. The fibrous fraction consists of cellulose and lignin components. Using paper, additional cellulose will be introduced into the system.

Further experiments will be focused on the improvement of the overall biodegradation of faecal material and the cellulose. Pre-treatment methods of the faecal material to improve the biodegradation efficiency will be selected based on a literature review.

Previous biodegradation experiments were performed in conditions at which the production of methane was not inhibited. In the Melissa-concept production of methane needs to be avoided. A concept was proposed whereby methanogenesis is inhibited by a high ammonium concentration in the reactor. Preliminary tests proved that methanogenesis occurred when the ammonium concentration in the reactor is higher than 8 g/l (TN26.3). The influence of increasing the ammonium concentration on the process stability and the biodegradation efficiency of faecal material and cellulose will be investigated in detail by fed-batch experiments.

Biodegradation experiments were performed at a thermophilic temperature equal to 55 °C to enhance the destruction of pathogenic organisms present in the faecal material. Further research on this topic is needed to collect representative data.

This technical note (TN34.1) contains following topics:

- Overview and description of the work package
- Review and trade-off of the literature concerning additional treatment of the faeces to improve the biodegradation.

Preliminary modelling of the biodegradation in order to calculate the conversion efficiency of the faecal material and the cellulose

2. WORK PACKAGE

The work package for the period between March '96 and March '97 is schematically presented in this topic.

The major points of interest are:

- Biodegradation efficiency of cellulose and faecal material combined with cellulose in methanogenesis inhibiting and non-inhibiting conditions
- Improvement of the biodegradation efficiency of the faecal material and cellulose by adding additional organisms or by pre-treatment of the feed
- Destruction of pathogenic organisms in thermophilic reactor conditions

2.1 Biodegradation experiments

2.1.1 Closed-bottle tests and cellulose biodegradation

A series of closed-bottle tests will be set up to determine the optimal conditions for cellulose degradation. Table 2.1 gives an overview of the different conditions at which the closed-bottle tests will be performed. The detailed description of the test and the results will be presented in TN34.2. The results of the closed-bottle tests fed-batch reactor tests are indicative for the design of the fed-batch reactor tests (See point 2.1.1).

Table 2.1 Overview of the experimental conditions for the closed-bottle tests

CLOSED BOTTLE TEST 1
Substrate: cellulose
Inoculum:
1. Inoculum containing a consortium of autochtonous bacteria present in faecal material
2. Inoculum containing a consortium of autochtonous bacteria present in faecal material with additional Clostridia strains (*Clostridium thermocellum* and *Clostridium thermosaccharolyticum*)
Temperature:
1. Mesophilic conditions (37 °C)
2. Thermophilic conditions (55 °C)
pH: between 7 - 7.5
CLOSED BOTTLE TEST 2
Substrate: cellulose
Inoculum: inoculum containing a consortium of autochtonous bacteria present in faecal material
Temperature: thermophilic conditions (55 °C)
pH: different pH levels : 6 - 7 - 8

2.1.2 Fed-batch reactor tests

Figure 2.1 gives a schematic overview of the fed-batch experiments that will be done. Three major parts can be distinguished in the experiments.

Experiment I

The biodegradation efficiency of cellulose by thermophilic bacteria will be tested in conditions at which methanogenesis is not inhibited.

The reactor content of a reactor containing a thermophilic inoculum with a consortium of autochtonous strains present in human faecal material (Reactor "RI4" TN 26.3) will be devided into two reactors. Reactor "TI" will contain the pure thermophilic inoculum and in reactor "TI+CI" additional cellulolytic Clostridia strains (*Clostridium thermocellum* and *Clostridium thermosaccharolyticum*) will be added. The reactors will be operated at thermophilic conditions and fed with powdered cellulose.

The results will be reported in TN 34.2.

Experiment II

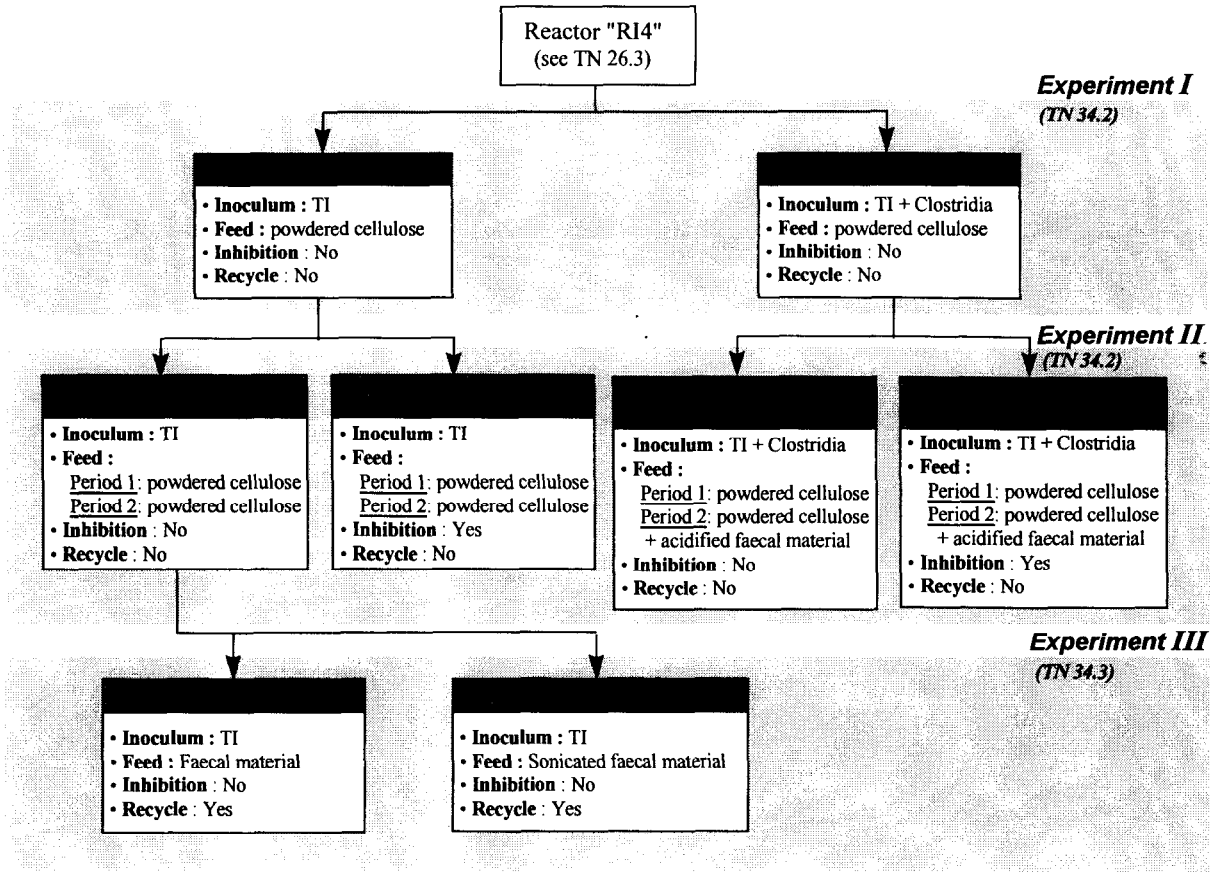
During the first period of the test, the biodegradation of cellulose by thermophillic bacteria in methanognesis inhibiting and non-inhibting conditions will be tested. The ammonium content of the reactors will be increased by addition of urea to inhibit the methanogenesis. The biodegradation of a mixture of cellulose and pre-acidified faecal material will be tested during the second period of the test. Figure 2.1. gives a schematic overview of the reactor configuration during "Experiment II".

The results will be reported in TN 34.2. Detailed information concerning pre-acidification will be reported in TN 34.3.

Experiment III

The effect of the sonication of the faecal material on the biodegrability will be tested. A part of the reactor content will also be centrifuged and the centrifuged cake will be fed again together with new faecal material to the reactor.

The results of the test will be presented in TN 34.3



TI : thermophilic inoculum

Figure 2-1 Overview of the fed batch reactor tests

2.1.3 Pre-acidification tests

Faecal material will be pre-acidified before it will be fed to reactor "TRc/TRcf" and "Trci/Trcfi" in the fed-batch experiment II.

2.2 Removal of pathogenic organisms

The effect of thermophilic anaerobic biodegradation of faecal material on the elimination of possible pathogenic organisms present in the faecal material will be tested in fed-batch experiment III. Results will be reported in TN 34.3.

3. MODELLING OF ANAEROBIC BIODEGRADATION

The use of the stochiometric model of Angelidaki et al (1993) for the calculation of the conversion efficiency

The calculation of the biodegradation efficiency of cellulose and faecal material is based on the measurements obtained in the fed-batch experiments. The main parameters that are used to calculate the mass balance are the organic matter content of the reactor feed, the amount and the composition of the produced biogas and the concentration of volatile fatty acids in the reactor.

The biodegradation efficiencies of the materials fed to the reactor were calculated taking into account

some boundary conditions. It was assumed that during the anaerobic biodegradation polymers where converted into volatile fatty acids. Besides volatile fatty acids, also other products like lactate or alcoholic compounds can be produced. The production of this compounds was not measured and not taken into consideration to calculate the biodegradation efficiency of the cellulose and the faecal material. In the Melissa-concept organic matter should be converted into volatile fatty acids and carbondioxide because this two components are useful for the Rhodospirillum compartment. Also lactate can be used by the Rhodospirillaceae. Yet, this component was not considered in the mass balance calculation. In the case that the production of biogas is not inhibited, the amount of produced biogas needs to be taken into account by calculating the biodegradation efficiency. Biogas is formed by the conversion of acetate by the methanogenic association. By the conversion of cellulose and faecal material also new biomass is produced. The transformation of organic matter into biomass can be considered as a non-efficient conversion in terms of the Melissa-concept.

Because only the produced volatile fatty acids and biogas are used to calculate the biodegradation efficiency, it is better to use the term conversion efficiency. This term indicates which part of the organic matter that is directly converted into volatile fatty acids and biogas. The conversion efficiency factor is an estimate for the part of the organic matter which may be converted into volatile fatty acids.

The biodegradation of organic matter can be summarised as follows :

First step: Hydrolysis

Organic matter --> Water soluble polymers

Second step: Acidogenesis

Water soluble polymers --> Volatile fatty acids

Third step: Acetogenesis

Volatile fatty acids --> Acetate

Fourth step: Methanogenesis

1) Hydrogenotrophic: Hydrogen gas --> Methane
2) Acetoclastic: Acetate --> Methane

Angelidaki et al (1993) presented a stochiometric model of the anaerobic biodegradation of cattle manure. In the model the several anaerobic biodegradation steps are described on a stochiometric basis. A theoretical mass balance was calculated based on this model and is summarised in Table 3.1.

Based on the theoretical stochiometric mass-balance (see Addendum 1) it can be calculated that in optimal conditions 1 gram organic matter is converted into 0.153 gram biomass and 0.907 gram of biogas which contains 51.5 vol% carbondioxide and 48.5 vol% methane. The production of biomass is not considered in the Melissa cycle as a usefull conversion. Taking this into consideration it can be theoretically calculated that the formation of one gram of biogas containing 48.5 vol% methane, corresponds with an effectieve conversion of 0.934 gram of organic matter.

A mixture of volatile fatty acids is accumulating when the methanogenesis is completely inhibited. The organic matter is converted into volatile fatty acids. One gram of organic matter is converted in 0.08 gram biomass, 0.741 gram volatile fatty acids (37 % acetic acid; 31 % propionic acid; 32 %

butyric acid) and 0.1874 gram biogas. This means that a production of 1 gram volatile fatty acids theoretically corresponds with an effective conversion of 1.24 gram organic matter.

Angelidaki and Ahring (1993) reported that the acetoclastic (acetate consuming) methanogens were more sensitive to ammonia stress then the hydrogenotrophic (hydrogen consuming) methanogens. When it is assumed that the volatile fatty acids are further converted into acetic acid and the produced hydrogen is converted into methane then is 1 g of organic matter converted into 0.122 gram of biomass, 0.755 gram of acetic acid and 0.194 gram of biogas containing 58 vol% carbondioxide and 42 vol% of methane. The methane is then produced by hydrogenotrophic methanogens. Based on this data it can be calculated that 1 gram of acetic acid formed corresponds with 1.16 gram of organic matter converted.

The composition of the biogas and the composition of the volatile fatty acid mixture measured in experimental conditions during the biodegradation of cellulose and faecal material are different compared to the theoretical model of the biodegradation of manure. To calculate the conversion efficiency for the different fed-batch reactors based on the experimental data, the theoretical conversion rates were simplified. It was presumed that the formation of one gram biogas corresponds with an effective conversion of 1 gram organic matter. The production of one gram of volatile fatty acid was presumed to correspond with 1 gram of organic matter converted. So, the calculated conversion efficiencies are indicative and an error on the reported efficiencies must be taken into consideration.

Table 3.1 Summary of the calculated mass balances based on the stochiometric model of Angelidaki et al. (1993)

ACIDOGENESIS			
Organic matter	1000.0	Biomass	80.2
Ammonia	11.7	Acetic acid	274.9
H2O	-	Propionic acid	227.7
		Butyric acid	238.8
		CO2	187.4
		CH4	-
		H2O	2.7
ACETOGENESIS Propionic acid --> Acetic acid			
Propionic acid	1000.0	Biomass	94.6
Ammonia	14.2	Acetic acid	757.6
H2O	76.4	Propionic acid	-
		Butyric acid	-
		CO2	142.8
		CH4	95.6
		H2O	-
ACETOGENESIS Butyric acid --> Acetic acid			
Butyric acid	1000.0	Biomass	83.9
Ammonia	12.6	Acetic acid	1289.2
H2O	164.3	Propionic acid	-
CO2	277.1	Butyric acid	-
		CO2	-
		CH4	80.9
		H2O	-
ACETOCLASTIC STEP			
Acetic acid	1000.0	Biomass	41.4
Ammonia	6.2	Acetic acid	-
		Propionic acid	-
		Butyric acid	-
		CO2	693.0
		CH4	252.0
		H2O	19.8
OVERALL MASS BALANCE			
Organic matter	1000.0	Biomass	153.1
Ammonia	22.0	Acetic acid	-
H2O	38.9	Propionic acid	-
		Butyric acid	-
		CO2	676.8
		CH4	231.0
		H2O	-
MASS BALANCE UP TO ACETOGENESIS			
Organic matter	1000.0	Biomass	121.7
Ammonia	17.5	Acetic acid	755.2
H2O	56.5	Propionic acid	-
		Butyric acid	-
		CO2	153.3
		CH4	41.1
		H2O	2.7

4. LITERATURE REVIEW OF PRE-TREATMENT METHODS

The anaerobic biodegradation of organic waste has a limited efficiency due to the presence of recalcitrant organic matter. This can be cell structures such as bacteria or vegetal compounds. The high content of bacterial biomass (1/3 of the organic matter) in human faecal material and the presence of fibrous materials limits the biodegrability of the faecal material. A literature review was performed to select adequate methods to improve biodegrability of organic material.

4.1 Overview

4.1.1 Catalytic liquefaction

Jungersen and Ahring (1994) reported the results of the experiments concerning the anaerobic digestion of cow manure pretreated by catalytic liquefaction.

During the anaerobic digestion of cow manure in anaerobic digesters, less than half of the organic material is degraded to methane and carbon dioxide. Catalytic liquefaction is a process whereby solid biomass is transformed into liquid products. The process proceeds under high pressure (60-250 bar) and temperatures of 300 to 360 °C. The liquefied manure consists of primary oils (30 - 60 %), water soluble organic matter (10 - 30 %), carbon dioxide and water.

The liquefied manure was digested in fed batch reactors. The optimum feed concentration for fed batch reactors under thermophilic (55 °C) conditions was 1 to 2 % liquefied manure of the total wet reactor volume. Under mesophilic conditions (37 °C) the optimal manure concentration was equal to 2 to 10 %. In optimal conditions the methane production rate for the thermophilic reactor was equal to 1.3 l methane per gram volatile solids of liquefied manure and for the mesophilic reactor equal to 0,25 l methane per gram volatile solids of liquefied manure. Continuous anaerobic digestion experiments at thermophilic conditions were also performed. The maximum methane production rate was obtained by a loading rate of 5 % liquefied manure and was equal to 1.5 l methane per gram volatile solids of liquefied manure. At a loading rate of 25 % the methane production was completely inhibited. During catalytic liquefaction a mixture of phenolic compounds are produced. The compounds can have a inhibitory effect, but at optimal feeding rates are they biodegraded for 85 %.

The authors expressed the performance of the anaerobic biodegradation of pig manure in terms of the volume of methane produced per gram of volatile solids of liquefied manure converted. There were no calculation to express the biodegradation efficiency expressed as a fraction of the initial organic matter content of the feed. At high loading rates, inhibition of methanogenesis occurred. The authors didn't report if there was still production of volatile fatty acids. Further research will be performed to select adequate methods to improve the biodegrability of faecal material

4.1.2 Thermal pretreatment

Li & Noike (1992) investigated the effect of thermal pretreatment on the mesophilic anaerobic digestion of waste activated sludge. The pretreatment temperatures ranged from 62 to 175 °C and the pretreatment times were 30, 60, 90 and 120 minutes. Waste activated sludge is mainly composed of microorganisms and the general composition has been known to be approximately 10 % carbohydrate, 50 % proteins and 10 % lipids and 30 % others including RNA and fibres. The effect of heat treatment on the solubilization of particulate organic matter is presented in Table 4.1. The COD removal efficiency during the anaerobic thermophilic digestion increased by the thermal pretreatment. The COD removal efficiencies at a retention time of 5 days increased from 28.3 % to 49.7 %, 54.9 %,

64.3 % and 59.0 % by a thermal pretreatment at 120 °C, 150 °C, 170 °C and 175 °C respectively. Table 4.2 gives the gas production per amount of COD in function of the residence time in the reactor and the pretreatment temperature.

Table 4.1 The characteristics of thermally pretreated activated sludge in function of the pretreatment temperatures and contact time

Parameter	Control	120 °C 30 min	150 °C 30 min	170 °C 60 min	175 °C 30 min
VSS/SS (%)	82.0	76.3	77.4	76.3	76.5
COD SR (%)	7.9	43.7	41.0	49.7	55.2
Carbohydrate SR (%)	6.0	41.8	48.4	46.6	50.8
Protein SR (%)	4.8	34.4	41.9	44.3	48.0
Lipid SR (%)	16.6	30.3	28.7	38.2	30.1
VFA (mg/l as COD)	166	1120	1230	2058	1912

SR: solubilization ratio

Table 4.2 Amount of gas per amount of feed fed to the reactor (ml/ g COD)

Retention time (days)	Control	120 °C 30 min	150 °C 30 min	170 °C 60 min	175 °C 30 min
1.5	74	144	163	170	162
3.0	77	152	181	197	171
5.0	108	159	208	223	216
10.0	-	185	216	235	212

4.1.3 Irradiation

Irradation with waves with a high energy content is technique that is used in food technology and environmental sanitation.

Masri (1995) reported the use of gamma radiation in feed technology to improve the digestibility of cattle feed. The digestibility can be improved through the reduction of crude fibre and the breakdown of polymers in agricultural residues rich lignocellulosic materials.

Three different types of feed blocks containing different proportions of molasses, poultry manure and agricultural residues such as weed bran or beet pulp were used in the test. The feed blocks were irradiated with gamma radiation in a doses from 0 to 150 KGy. Gamma irradiation had no effect on the total nitrogen, in vitro crude protein digestibility, acid detergent fibre, acid detergent lignin and cellulose. At a the optimal dose of 100 KGy the crude fibre content and neutral detergent fibre content was reduced with respectively 11 % and 20 %. The hemicellulose content was reduced for 35 to 57 %.

Gamma irradiation is also used to eliminate pathogenic organisms in hospital waste. The Thermorad process combines heat treatment with radiation (Icre et al., 1995). A dose of 5 KGy at 60 °C is sufficient to decrease pathogenic organism with a factor 10^5.

UV-irradiation can also be used to destroy recalcitrant components present in waste water streams which may not be biodegraded in anaerobic waste water treatment systems or which are toxic. UV light can be used in combination with TiO_2 catalysts (Tanaka & Ichikawa, 1993).

4.1.4 Sonication

The effects of sonication activated sludge were reported by King & Forster (1990). Sonication with power levels of 7.5 - 75 W caused disruption of the sludge flocs which increased with the intensity of the power. There was a relationship between the mean particle size and the sonic power. Sonication also released soluble carbohydrate and protein from the sludge. It appeared that there was a sequential release of different biopolymers from the sludge as the power was increased.

4.1.5 Pre-acidification of organic matter

A biological method to increase the degradation efficiency of the organic fraction of municipal solid waste was reported by Kübler and Schertler (1994). The BTA-process comprises pre-treatment of incoming waste followed by a three-phase anaerobic biology consisting of acidification, solids hydrolysis and methanisation. By using this system a remarkable biodegradation of cellulose was noticed. Figure 4.1 gives a schematic presentation of the process.

The system ensures a significant degradation of cellulolytic material. This is due to the fact that the concentration of soluble compounds generated during hydrolysis is kept low in the system. This is necessary because it is proven by Buchholtz & Arntz (1994) that the presence of soluble compounds inihibits significantly hydrolysis.

During the spontaneous acidification about 550 liter biogas was produced,per kg of organic material. About 55% to 73 % of the dissolved COD was acidified during the pretreatment. The acidified material is dewatered to remove the soluble organic matter and the cake is diluted again with effluent of the methane reactor. This material is brought in the hydrolysis reactor and further hydrolysed. The optimal residence time in the hydrolysis reactor was about 4 days. During this period about 84 % of the remaining organic matter was biodegraded.

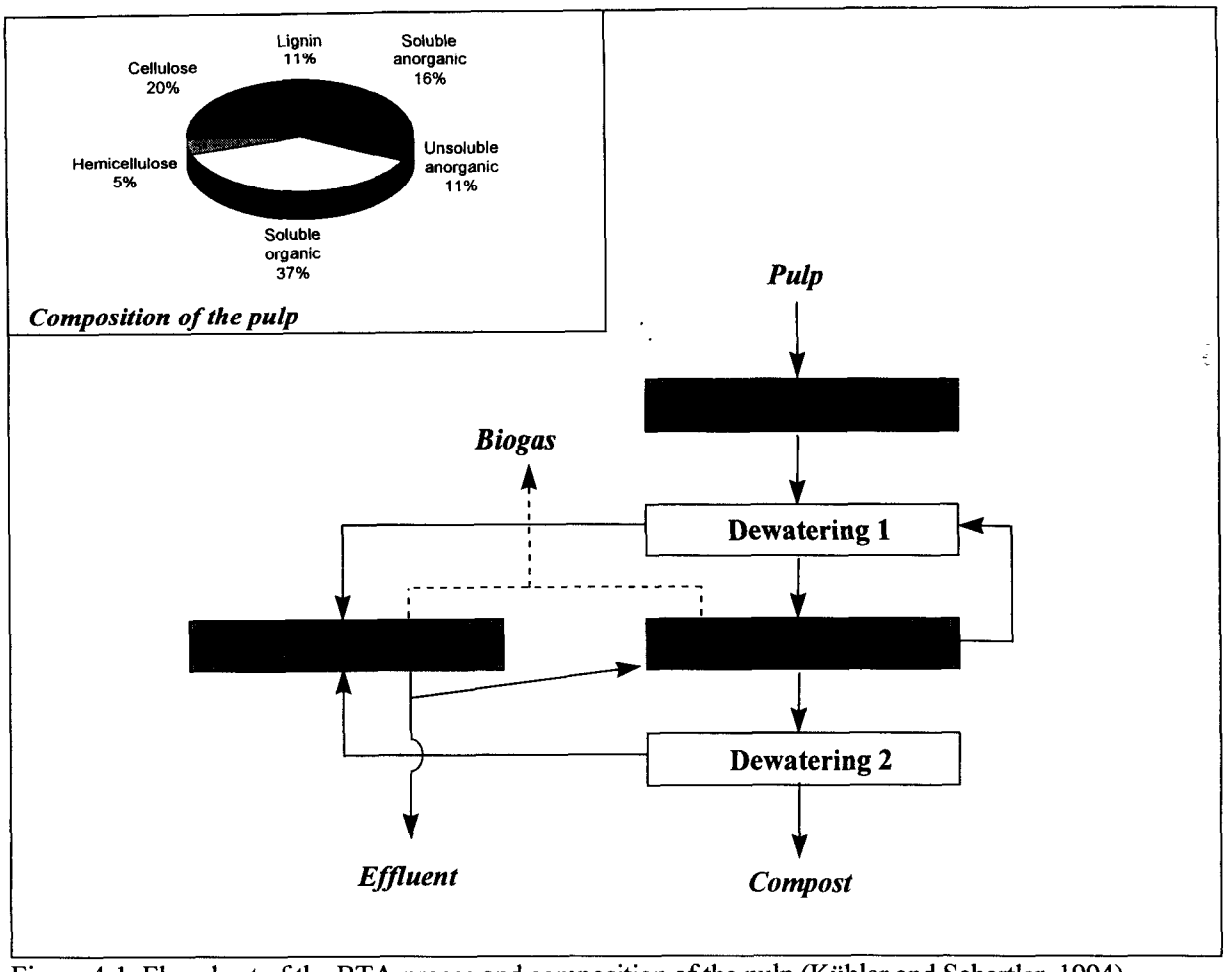


Figure 4-1. Flowsheet of the BTA-proces and composition of the pulp (Kübler and Schertler, 1994)

4.2 Discussion

It appears that **liquefaction** of human faeces could be a method to improve the biodegradation efficiency of the material. Jungersen and Ahring (1994) reported a methane production equal to 1.5 l per gram volatile solids of liquefied manure at thermophilic conditions. This high value indicates a good conversion efficiency. The drawback of the method is the need for adequate technology to perform the liquefaction process in a safe way because very high pressure is needed. Li & Noike (1992) reported that **thermal pretreatment** of activated sludge at a temperature equal to 170 °C during 60 minutes resulted in COD removal efficiencies equal to 65 % by mesophilic anaerobic digestion (33 °C). This could be a valuable method to improve the biodegrability of faecal material. Yet, high temperatures are needed and still adequate technology is needed. **Irradiation** is a technique used in feed industry to improve digestibility of feed supplements with a high fibre content. There were no applications of irradation techniques to improve the biodegradation of manure or sludge reported in literature yet. Yet, in extra terrestrial conditions a lot of irradation is available. The concept of irradiation of faecal material can be taken into consideration to improve the biodegradability of faecal material. King & Forster (1990) reported that **sonication** of activated sludge had a destructive effect on sludge flocs. There was no information found in literature concerning the sonication of organic material to improve the biodegradability. The use of sonication was selected to perform an experiment in which the effect of sonication on the biodegradability of faecal material will be tested. This pretreatment method was selected because it is a relative simple technology and not a lot of literature information on this topic is available (See item 2..1.2. Experiment III). Kübler and Schertler (1994) reported the result of **a biological method** to improve the biodegradation of organic material. This method was selected to use in the biodegradation

experiments because of the fact that it relatively simple and complete biological process (See item 2..1.2. Experiment II). In the experiments of WP34 sonication and pre-acidifacation as a method to pre-treat the faecal material was tested. Thermal heating will be tested in a next work package.

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