EPAS n.v.

Eco Process Assistance

IIC-Universiteit Gent
Technologiepark 3 - 9052 Gent (Zwijnaarde)

Tel. (09) 241.56.18
Fax (09) 221.82.18

MELISSA

Memorandum of understanding
ECT/FG/MMM/97.012

ESA/ESTEC PO : 163031

TECHNICAL NOTE : 41.3

Introduction of a STAGE II compartment

Version : 1
Issue : 0

Demey D., Van Meenen P., Hermans V.

November 1998

DOCUMENT CHANGE LOG

Version	Issue	Date	Observation
1	0	10/08/98	Original version
2	0	30/10/98	Original version
1	0	26/11/98	Final version

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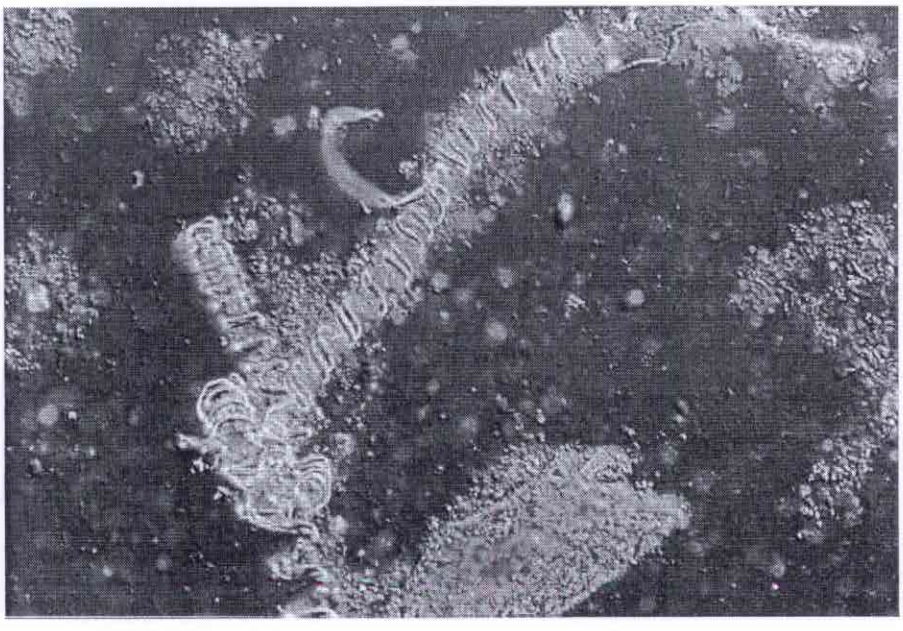
1. Introduction

The biodegradation efficiency of faecal material by an inoculum of autochtonous bacteria at thermophilic conditions (55°C) and a low pH (6.5) was equal to about 30 %. It appeared that proteins were biodegraded for about 70 % and non-protein material, containing a fibrous material for only 10 %.

An important part of faecal material consists of bulk material which is formed by difficult digestible plant material taken up by the food. Dietary fibre is usually defined as the dietary fraction which is not digested by the endogenous enzymes of the digestive tract. The major components of dietary fibres are insoluble plant cell walls which are composed by polysaccharides, lignin and wall proteins.

Based on the low biodegradation efficiencies obtained during the fermentation experiments it can be stated that a considerable amount of fibrous material is present in the reactor. This hypothesis is supported by microscopic examination of the reactor content. Cell wall components can be clearly detected in the samples as illustrated in Picture 1-1.



Picture 1-1. Presence of plant cell wall components in the anaerobic reactor

This technical note reviews information on the slowly biodegradable fraction of the faecal material. Based on this information proposals are made to expand the liquefying compartment with a second compartment to treat the slowly biodegradable fraction.

2. Dietary fibres

2.1 Definition

Dietary fibre is usually defined as the dietary fraction which is not digested by the endogeneous enzymes of the digestive tract. For this reason, it can be assumed that the parameter can also give a good indication of the resistant organic fraction present in faecal material.

2.2 Determination

Two groups of methods for the determination of dietary fibre can be distinguished. A first approach is the determination by means of gravimetric methods. Non-fibre components are removed by enzymatic treatment from the sample. The separated fractions are weight and the results are expressed as non-starch polysaccharides plus lignin. A second group of methods is based on the determination of the neutral sugar and uronic acid content after enzymatic removal of starch and acid hydrolysis. The monomeric components are analysed by means of a gas chromatograph or spectrophotemetrically. The amount of individual components is summed to obtain the total dry fibre content. Total dietary fibre can be split in water soluble and non-soluble dietary fibre. Non-soluble fibre is defined as the fibrous material that dissolves in water at 100°C and precipitates in alcohol. Starch-like components are not enclosed in the dietary fibres.

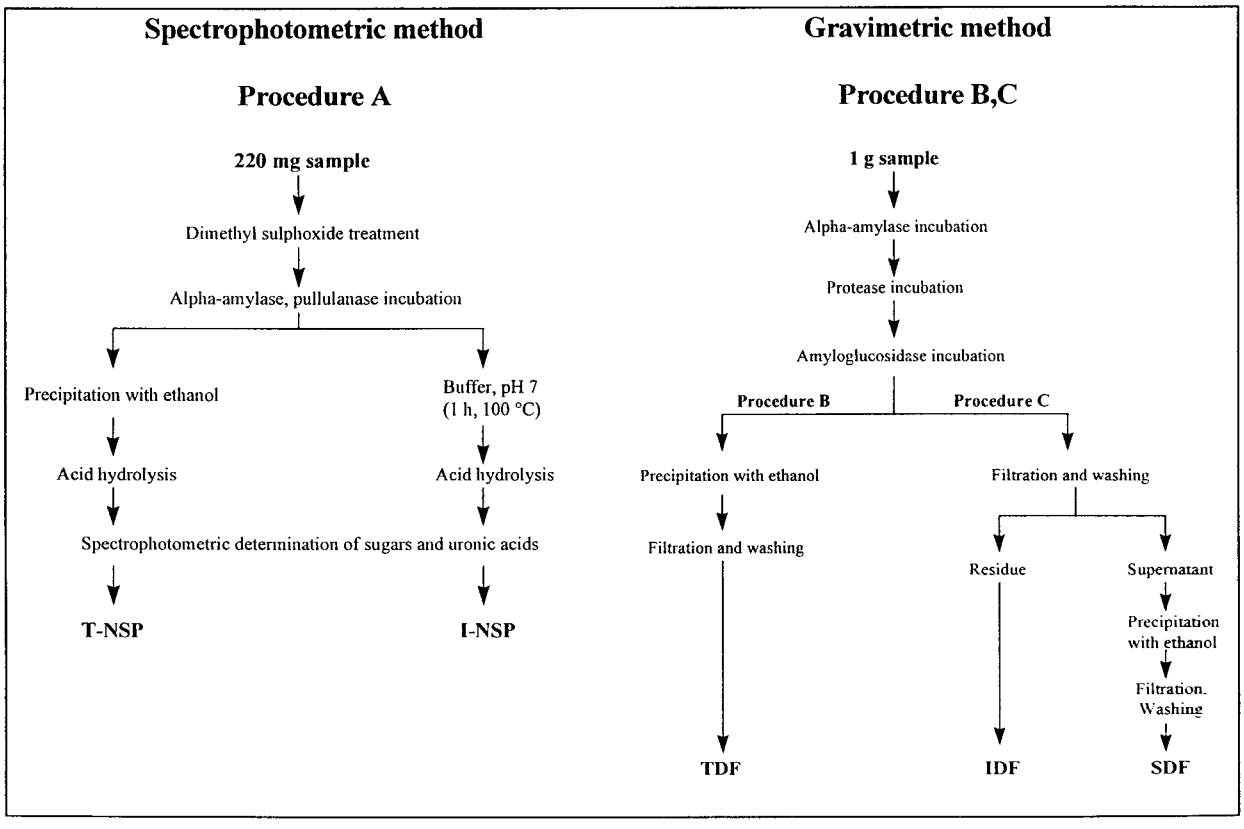


Figure 2-1. Schematic presentation of the analysis of fibres with the spectrophotometric method and the gravimetric method (Goni et al, 1989)

Both methods can be illustrated by an analysis of the fibre content of apple pulp (Goni et al, 1989). The samples were grind after freeze-drying and analysed as schematically presented in Figure 2-1. Table 2-1 gives the result of the dietary fibre composition determined by means of

the spectrophotometrically determination of neutral sugars and uronic acids obtained after removal of starch and acid hydrolysis. Table 2-2 gives the results of the gravimetric method. Spectrophotometric analyses of neutral sugars and uronic acids were also determined on the obtained residues. Treatment of IDF and TDF with HCl/BuOH show the presence of condensed tannins. These compounds form complexes with protein and could be considered as fibre constituents because they escape digestion. Additionally lignin was determined by the Klason method (see item 3.2.2) on the total dry fibre (TDF) and insoluble dry fibre fraction (IDF) and corrected for the amount of condensed tannins and protein content of the Klason lignin residue. Comparing the two methods learns that the by means of the gravimetric method and additionally lignin and tannin determination, the total dietary fibres including polysaccharides, lignin and condensed tannins can be determined.

Table 2-1. Content of dietary fibres of apple pulp determined spectrophotometrically (procedure A) as non-starch polysaccharides (NSP) (% dry matter) (Goni et al, 1989)

	Total NSP	**Insoluble NSP**	**Soluble NSP[1]**
Neutral sugars	32.8	28.4	4.40
Uronic acids	15.5	5.56	9.94
Dietary fibre [2]	48.3	33.96	14.34

[1] Soluble NSP = Total NSP - Insoluble NSP
[2] Dietary fibre = Neutral sugars + Uronic acids

Table 2-2 Content of dietary fibres of apple pulp determined gravimetrically (% dry matter) (Goni et al, 1989)

	Procedure B		**Procedure C**	
	Total DF	Insoluble DF	Soluble DF	Total DF
Experimental values				
Dietary fibre	66.38	51.33	14.18	65.51
Klason lignin + phlobaphenes	20.01	-	-	-
Proteins of Klason lignin + phlobaphenes	1.93			
Condensed tannins	3.04	3.10		
[1]Neutral sugars	32.60	27.50		
[1]Uronic acids	15.45	5.75		
Calculated values				
Polysaccharides	48.10	33.25		
Lignin	15.04	-		
Dietary fibre	68.11	-		

[1]Determined spectrophotometrically on the obtained residues
Polysaccharides = neutral sugars+ uronic acids
Lignin = (Klason lignin + phlobaphenes) - (proteins of Klason lignin + phlobaphenes) - condensed tannins
Dietary fibre = polysaccharides + lignin + (proteins of Klason lignin + phlobaphenes) + condensed tannins

For the determination of dietary fibre, 10 g of dry solids is necessary. For the MELISSA compartment it means a volume of about 1 litres. Another laboratory only needs 200 ml, but because of the high costs, frequent analyses can't be possible.

2.3 Analysis of the polymeric composition

The cell wall monomers are indicative for the presence of different polymeric structures in fibrous material. The occurrence of the neutral sugars is indicative for the type of polymeric material present in the sample. This means that it is possible to predict the occurrence of different polymeric substances by determination of the released monomers after acid hydrolysis. Table 2-3 gives an overview of the typical monomers released when destructed by acid hydrolysis. Uronic acid arises from pectic substances. Pectic polyssacharides are the primary cell wall polymers found in the parenchyma of dicotyledonous plants and some monocotyledonous plants. Uronic acid can also be produced during hydrolysis of hemicellulose. Glucose is mainly generated during the degradation of cellulose. Cellulose is present in roughly equal to slightly lower concentrations than pectic substances in parachymatous tissues of dicotyledons. Arabinose and galactose are present as arabinan, galactan and arabinogalactan side chains of pectic substances. Hemicellulose is the major source of mannose and xylose. Fucose is a component of hemicellulosic xyloglucans. Acetyl-groups are derived from xylose residues from xylans and also pectic substances.

Table 2-3 Monomers released from polymers during acid hydrolysis

Monomers	**Polymeer**			
	Cellulose	Hemicellulose	Pectine	Xylans
Glucose	+++	+		
Mannose		+++		
Xylose		+++		
Fucose		+++		
Arabinose		+	+++	
Galactose		+	+++	
Rhamnose			+++	
Uronic acis		+	+++	
Acetyl-groups			+	+++

+++ abundant ; + present

2.4 Polymeric substances in vegetables

Table 2-4 gives the composition of different vegetables analysed by Bourquin et al. (1992). It appeared that uronic acids were the monosaccharides present in greatest quantities in all vegetables except cumcumber. This indicates that the major part of the fibres was pectine. Glucose was the second most abundant monosaccharide constituent of all substrates except cumcumber. This means that cellulose was an important polymer. Arabinose and galactose were present in variable concentrations for the different vegetables. The low concentrations of mannose and xylose, which are the major components of hemicellulose, indicate that the amount of hemicellulose in the cell wall of the investigated vegetables is small in comparison with pectin substrates and cellulose.

Table 2-4. Composition of different vegetables in terms of fibres (Bourquin et al, 1993)

	Substrate							
	Broccoli	Carrot	Cauli flower	Celery	Cucum ber	Lettuce	Onion	Radish
				g/kg as is				
Dry Matter	112.7	124.9	83.9	43.7	29.6	34.2	51.6	43.1
Ethanol insoluble residue	72.4	49.9	48.5	18.8	9.6	13.5	26.1	18.0
Total dietary fibre residue[1]	53.8	36.5	36.5	17.7	7.8	12.2	22.3	16.3
Total dietary fibre[2]	34.9	29.2	24.8	13.5	5.8	8.9	17.6	12.5
g/kg dry matter								
Total dietary fiber residue	477.0	292.0	435.0	405.0	264.0	357.0	432.0	378.0
Total dietary fibre	310.0	234.0	296.0	309.0	196.0	260.0	341.0	290.0
Non-starch polysaccharides[3]	223.0	176.0	223.0	217.0	133.0	174.0	256.0	209.0
g/kg total dietary fibre residue								
Organic matter	871.0	859.0	888.0	844.0	847.0	840.0	847.0	852.0
Crude protein	222.0	59.0	207.0	80.0	101.0	114.0	55.0	84.0
Total dietary fibre	649.0	800.0	681.0	764.0	746.0	726.0	792.0	768.0
Cell wall monomers (anhydrous)								
Arabinose	84.3	61.4	94.2	39.1	19.2	14.4	12.4	25.1
Fucose	3.0	TR4	3.6	2.0	2.0	2.4	2.8	2.5
Galactose	47.3	107.2	49.3	39.3	85.0	24.1	149.4	23.0
Glucose	133.3	164.2	146.3	179.5	174.6	161.8	161.7	181.6
Mannose	14.2	15.5	15.7	18.3	21.1	17.5	9.0	17.6
Rhamnose	TR[4]	1.8	TR	TR	1.4	5.2	3.2	6.9
Xylose	21.3	7.9	23.1	21.4	36.4	24.5	16.4	20.9
Uronic acids	164.5	244.8	181.2	237.1	164.6	238.7	237.3	275.1
Acetyl	12.9	17.4	16.3	15.6	13.4	13.7	10.2	15.5
Klason lignin	49.5	24.3	24.2	43.2	46.2	76.6	22.6	41.8

Crude residue not corrected for ash and protein.
[2]Corrected for residual ash and protein.
[3]Calculated as: Sum of neutral monosaccharide and uronic acid contents in total dietary fider residues (g/kg) total dietary fiber residue content of dry matter (g/g).
[4]Trace amounts detected.

3. Biodegradation of fibrous material

3.1 Biodegradation of cellulose

Cellulose is an important element of plant material and consists of a long chain of glucose molecules linked to one another with β (1-4) glycosidic bonds. Cellulose can be present in different forms: christalline cellulose has chains which are highly hydrogen bond-ordered; amorphous cellulose has partially degraded and partially hydrated chains; water soluble cellulose has completely hydrated and water soluble chains. Cellulose is one of the most abundant organic substances in the environment and can be attacked by a variety of micro-organisms which are characteristic for certain environmental conditions. The relative simplicity of the cellulose molecule means that only a small number of enzymes is required to biodegrade the component. Yet, cellulose in plant material is cross-linked with hemicellulose and lignin creating a complex structure that is not always easy to biodegrade.

3.1.1 Anaerobic biodegradation

Anaerobic thermophilic processes are well known in the processing of organic solid waste and waste waters. The organisms found in thermophilic anaerobic systems are members of the genus Clostridia. Especially *Clostridium thermocellum* is a well known and well studied species. Clostridia species are producing extracellular cellulases which can effect total solubilization of crystalline cellulose. When raw plant material is used as a cellulose source it appeared that the conversion rates were not always that high as expected. Pretreatment of wood at high temperature in acid conditions for example increased the biodegradation by *Clostridium thermocellum*. Recently, the occurrence of highly thermophilic organisms (70 °C) are reported, but up to know little fundamental information, is available.

Anaerobic digestion of cellulose at mesophilic conditions is of major importance in the rumen. The rumen environment is characterised by a low elelctronic potential (-150 to -350 mV), a temperature between 38 °C and 42 °C and a buffered medium between pH 5.5 and 7. Important genera are Fibrobacter, Butyrivibrio, Eubacterium and Ruminococcus. *Fibrobacter succinogenes* ferments only glucose, cellobiose and cellulose. Primary end products are acetic and succinic acid. *Ruminococci flavefaciens* ferments cellulose and cellobiose. Some other Ruminococcus utilize only polysaccharides for growth (i.e., cellulose, xylan and pectin). Principal end products are acetic, formic and succinic acids. Ruminococci need NH_3 and accumulation of reducing sugars when grown on an excess of cellulose. The importance of Butyrivibrio and Eubacterium as cellulolytic bacteria in the rumen fluid is doubtful since their low cellulose digestion (Burk, Ohio State University). The ability of the cellulolytic rumen bacteria to digest or solubilize purified cellulose is shown in Table 3-1. The conditions of the three studies varied, but in general *F. succinogenes*, *R. albus* and *R. flavefaciens* can solubilize a considerable amount of powdered cellulose. Only *F. succinogenes* could solubilize appreciable amounts of undegraded cotton fibre. Table 3-2 present data on solubilization of cellulose from intact forages. Although the data are limited, *F. succinogenes* appears able to solubilize more cellulose than the ruminococci.

Table 3-1Solubilization of purified cellulose by the principal cellulolytic bacteria in the rumen

Organism	*% solubilized*[a]		*% solubilized*[b]	*% solubilized*[c]
	Cellulose powder	*Cotton fibre*	*Cellulose powder*	*Cellulose powder*
F. succinogenes	88	97		69
R. albus	88.88	10.4	78.79	64
R. flavefaciens	72.90	0.55	38.39	63
B. fibrisolvens			15.20	
E. cellulosolvens			0.0	

[a] Data from Halliwell et al., 1963
[b] Data from Gylswyk et al., 1971
[c] Data from Hiltner et al., 1983

Table 3-2 Solubilization of cellulose from intact forages by cellulolytic rumen bacteria

Organism	*% Solubilization*[a]		*% solubilization*[b]
	Bromegrass	*Alfalfa*	*Teff hay*
F. succinogenes	79.81	51.52	
R. albus	57	43	43.56
R. flavefaciens	49-65	20-48	39.66
B. fibrisolvens	15	6	10-37
Clostridium sp.			10.11

[a] Data from Dehority et al., 1967
[b] Data from Kock et al., 1969

Beside bacteria also ciliate protozoa are present in the rumen and recently identified anaerobic fungi (*Neocallimastix*) with specific cellulolytic activity. Species of bacteria found in the rumen are also identified in sludge digestors, but up to now, no presence of anaerobic fungi in digestors is reported. Cellulose and hemicellulose is well degraded by anaerobic mesophilic species but lignocellulolic activity is not proved yet.

3.1.2 Aerobic biodegradation

Aerobic biodegradation processes of solid organic materials are well known in aerobic composting systems. The composting process is characterised by a temperature evolution from thermophilic to mesophilic. The micro organisms most abundant during the beginning of the process are bacteria of the Genera Bacillus, Thermus and Thermomicrobium. During the thermophilic period Actinomycetes are present. The most important Genus is Thermomonospora. Actinomycetes are producing cellulases, but also hemicellulases, pectinases and lignolytic enzymes. Thermomonospora species are the dominant Actinomycetes that sometimes can be found in compost piles. Thermomonospora needs biotin and thiamine combined with a carbon source for optimal growth. Temperature optimum is equal to 7.5-8.0 and pH optimum is equal to 7.5-8.5. At the thermophilic stage, also thermophilic fungi are present. At the end of the composting process mesophilic fungi are dominant.

The change in lipids, sugars, soluble polysaccharides, cellulose and lignin was determined during a 45-day incubation of ryegrass straw (Horwath, 1996). All the straw components declined during the low-temperature treatment (Table 3-3). The initial increase in lignin and soluble polysaccharides indicates microbial production of membranes and extracellular polysaccharides. In the high-temperature, the straw chemical fraction were mineralized more rapidly and completely than in the low-temperature treatment.

Table 3-3 Mean change in chemical fractions (g/kg straw) and % degradation during low- and high-temperature incubation (Horwath, 1996)

Day	*Lipids*	*Sugar*	*Soluble polysaccharides*	*Cellulose*	*Lignin*
Low temperature					
1	33.1	33.0	17.1	562.1	121.5
3	33.7	13.3	21.8	542.0	127.9
7	38.3	9.8	19.0	481.9	125.6
12	31.5	8.2	11.4	450.8	125.1
20	26.9 (18.73 %)	7.3 (77.9 %)	11.1 (35.1 %)	381.8 (32.1 %)	122.2 (-0.6 %)
30	26.4	7.0	8.9	312.9	114.1
45	23.5 (29.0 %)	6.9 (79.1 %)	10.1 (40.936 %)	297.8 (47 %)	113.0 (6.99 %)
High temperature					
6	43.7	12.6	13.7	513.8	119.1
8	38.0	7.5	12.3	522.2	119.5
12	35.0	17.9	9.0	315.8	109.1
17	30.4	16.7	8.9	289.0	106.0
25	26.0 (21.45 %)	11.6 (64.8 %)	6.2 (63.7 %)	219.4 (61 %)	102.8 (15.4 %)
30	25.6	6.1	6.1	185.5	102.4
45	18.7 (43.5 %)	9.7 (70.6 %)	5.6 (67.3 %)	213.8 (62 %)	89.0 (26.7 %)

Cellulose degradation by fungi is well known and studied in detail because of their major importance in the eco-sytem and particularly for their commercial use. Fungi produce, in contrast with bacteria, extracellular cellulases which can be purified from the culture. Important genera are Trichoderma, Fusarium, Penicillium and Aspergillus.

3.2 Biodegradation of lignin

3.2.1 Characteristics of lignin

Lignin is an important complex polymeric plant cell wall component consisting of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds. This complex structure obstructs detailed chemical characterisation. Few organisms are able to biodegrade lignin by extracellular lignase and manganese peroxidases. White-rot fungi are able to biodegrade lignin as well Actinomycetes. Lignin degradation is primarily an aerobic process and in the anaerobic environment lignin can persists for very long periods. Lignin is the most recalcitrant component of plant material. Due to the physical linkage with other cell wall components lignin restricts also the enzymatic attack of these other components resulting in a lower biodegradability.

3.2.2 Determination of lignin

Lignin determinations are in many environmental and nutritive studies performed by means the Klason method. The total dietary fibre content needs to be removed from the sample and is further treated by adding 12 M H_2SO_4. The mixture is further digested at 30 °C for 1 hour and terminated by dilution with water to 0.358 M H_2SO_4.The digested samples are filtered on a glass fibre filter and weighed after 72 h drying at 105 °C. it should be noticed that alos not lignin components such as tannins may be measured by this method. Correction for tannins is possible by reading absorbances at 550 nm of anthocyanidin solutions obtained after treatment of the dietary fibre sample with 5 % HCl-BuOH for 3 h at 100 °C.

3.2.3 Anaerobic biodegradation of lignin

Biodegradation of lignin in anaerobic conditions is low. Chandler et al. (1980) proposed a mathematical correction for the bioavailability of an organic substrate based on it's lignin content. A linear relationship was established based on anaerobic digestion data of different materials by 40 days retention time. Chandler's formula gives reasonable accuracy for materials with a relatively low lignin content. Based on recent analysis Van Soest (1996) proposed a log linear relationship between the anaerobic biodegradability of plant material and the lignin content that can be applied with more accuracy.

Chandler (1980) :

$$F_b = 0.83 - (0.028 \cdot lignin_{\%VS})$$

Van Soest (1996) :

$$F_b = 100 - 5.41 \cdot (lignin_{\%DF})^{0.76}$$

F_b: biodegradble fraction
$lignin_{(\%ofVS)}$: lignin content as percentage of volatile solids
$lignin_{(\%DF)}$: lignin content as procent of dietary fibres (= cellulose+hemicellulose+lignin)

Ruminal fungi may solubilize lignin, but there is no evidence that they are able to cleave the intermonomer bonds in lignin or to utilize lignin as a carbon source. The study of Mc Sweeney investigated wether the anaerobic fungus *Neocallimastix patriciarum* could cleave lignin bonds and linkages between lignin and polysaccharide.

3.2.4 Aerobic biodegradation of lignin

Based on literature reports it can be stated that lignin can biodegrade significantly in aerobic systems, but with a quite variable efficiency. Horwath and Elliot (1996) studied the biodegradation of ryegrass straw in mesophilic (25 °C) and thermophilic (50 °C) aerobic incubations. Lignin was decreased by 10 % and 29 % in the low- and high temperatures incubations respectively. The fraction of the elements O and N increased in the lignin residue and the fraction of C and H decreased. This indicates that the lignin fraction was oxidised during the biodegradation period. During this oxidation the side chains are splitted and oxidative ring cleavage occurs to form carboxylic acid groups. Hammouda and Adams (1989) measured lignin degradation ranging from 17 % to 53 % in grass, hay and straw during 100 days of composting. Tomati et al. (1995) measured a 70 % reduction in the lignin content of olive waste compost after 23 days under high moisture (65-83 %) thermophilic conditions. After this initially high decomposition rate under thermophilic conditions, no further reductions in lignin content during the subsequent 67 days under mesophilic conditions. Adding small quantities of nitrogen to woody materials can increase lignin degradation rates. Two kind of wood (Alder and Hemlock) were incubated for two weeks with a white-rot fungi at 39-40°C. Table 3-4 shows the results of the impact of the addition of different amounts of nutrient nitrogen on Hemlock and Alder wood. Adding only 0.12 % nitrogen (dry weight basis), lignin degradation in alder pulp increased from 5.2 % to 29.8 % (Yang et al., 1980).

Table 3-4 Influence of Nutrient Nitrogen on Pulp component decomposition in two weeks (Yang et al, 1980)

		Percent decomposition		
	Added nitrogen (% dry pulp basis)	*lignin*	*carbohydrates*	*total weight*
Hemlock	0	2.2	<0.1	1.2
	0.12	3.9	7.3	6.0
	0.24	3.8	6.4	5.1
	1.20	2.7	8.0	6.3
	2.40	1.2	5.5	4.2
Alder	0	5.2	3.4	4.1
	0.12	29.8	21.0	22.8
	0.24	30.9	26.6	25.2
	1.20	30.4	31.0	28.0
	2.4	12.3	14.6	13.1

The impact of lignin degradation on the biodegradability of the remaining carbon has not been extensively researched. In one of the few studies which might provide such insight, Latham (1979) measured a 5 to 11% increase in anaerobic digestability of barley straw after 3 to 4 week aerobic incubations at 30°C with various pure cultures of white-rot fungal species.

3.2.5 White rot fungi and Actinomycetes

Haertig and Lorbeer (1993) stated that the necessity of mycelial growth and the formation, secretion and extracellular action are main requirements for a successful microbial attack on polymeric lignin. Especially two groups of organisms are capable to biodegrade lignin: White-rot fungi and Actinomycetes. White rot fungi are fungi from the Basidiomycetes group which produce a wide range of necessary enzymes such as peroxidase, manganese(II)peroxidase, cellobiose:quinone oxidoreductase (CBQ) and cellobiose oxidase (CBO) to biodegrade lignin. Conditions which favours the growth of white-rot fungi, including adequate nitrogen, moisture and temperature, all appear to be important in encouraging lignin decomposition, as does the composition of the lignocellulosic substrate itself. Another group are the Actinomycetes which are classified as bacteria. Actinomycetes produce also a mycelium like structures and enzymes to biodegrade lignin.

3.2.6 Pretreatment of lignocellulosic material

Biodegradability can be enhanced by pretreatment of lignocellulosic materials, including acid or alkali treatment (Jackson, 1977; Van Soest, 1994), ammonia and urea (Basaglia et al., 1992; Van Soest, 1994), physical grinding and milling (Ladisch et al., 1983), fungal degradation and steam explosion (Sawada et al, 1995), and combined alkali and heat treatment (Gossett et al., 1976). Gharpuray et al. (1983) examined several of these pretreatment options individually and in combination. In this study wheat straw was used as the lignocellulosic biomass. The enzymatic hydrolysis is a heterogeneous reaction and is therefore influenced by the structural features of the substrate such as crystallinity, lignin content and surface area. An increase in surface area and a decrease in lignin content and crystallinity enhance the hydrolysis rate. The effect of pretreatment on structural features and composition of substrate is shown in Table 3-5. All chemical pretreatments led into an increased surface area and hydrolyis rate. The cristallinity index declined with ball-milling time. Physical pretreatment are relatively less effective in enhancing the hydrolysis rate as compared to the chemical pretreatments.

Cellulose hemicellulose and lignin contents of wheat straw which were subjected to a variety of chemical pretreatments are tabulated in Table 3-6. Ethylene glycol extracted most of the hemicellulose content but enriched the cellulose content.

Table 3-5 Effect of single pretreatment on structural features and hydrolysis rate (Gharpuray et al., 1983)

Type of pretreatment	*Specific surface area (m^2/g)*	*Cristallinity index*	*Lignin content (%)*	*Relative extent of hydrolysis after 8 h*
Caustic soda	1.7	53.3	5.29	8.00
Peracetic acid	1.7	28.4	2.60	13.25
Ethylene glycol	2.9	63.6	3.27	6.83
Ball-milling				
4 h	2.3	23.7	11.53	4.0
8 h	1.8	54.5	11.53	4.0
16 h	1.9	17.5	11.53	3.6
24 h	2.0	19.4	11.53	4.4
Standard *	0.64	69.6	11.53	1.0

* untreated substrate

Table 3-6 Effect of chemical pretreatment on composition of wheat straw (Gharpuray et al., 1983)

Type of pretreatment	*Cellulose (%)*	*Hemicellulose (%)*	*Lignin (%)*
Caustic soda	49.33	19.32	4.05
Peracetic acid	30.74	14.56	2.05
Ethylene glycol	51.79	1.39	4.68
Standard *	41.15	26.10	13.05

* untreated substrate

4. Proposals

4.1 Introduction of an additional compartment

A major part of the non-biodegradable fraction of human faecal material consists of fibrous material. It was the intention to use a mixed culture of Clostridia bacteria (*Clostridium thermophilum* and *Clostridium thermossacharolyticum*) together with *Coprothermobacter proteolyticus* to inoculate the liquefying compartment of the Melissa loop. It appeared that the inoculum was able to biodegrade synthetic substrate, but not a complex substrate such as human faecal material.

The most recalcitrant components are lignin and cellulose in a form that is difficult to attack by the anaerobic bacteria of the first compartment. This study indicated that indeed this kind of components are hardly biodegraded in anaerobic conditions. Aerobic conditions are needed to stimulate the growth of micro organisms capable to biodegrade fibrous materials. Especially White rot fungi and Actinomycetes can biodegrade lignocellulytic components.

The introduction of a additional second compartment is proposed in which the fibrous components of the faecal material may be converted. After further cleavage of fibrous material

by fungi or Actinomycetes, the final product can be recycled to the anaerobic liquefying reactor. Latham (1979) measured a 5 to 11 % increase in anaerobic digestability of barley straw after 3 to 4 week aerobic incubations at 30°C with various pure cultures of white-rot fungal species.

A second aerobic reactor can be inoculated with Actinomycetes or Basidiomycetes obtained from their natural ecosystems such as aerobic composting piles, soils, forest litter,... These organisms can be isolated and grown on a specific aerobic medium 1/3 strenght PDARP. This medium, especially used for culturing fungi, contains Potato Dextrose Agar (6.5 g), Bacto Agar (5.0 g), distilled water (500 ml), Rifampicin (15 mg) in Methanol (10 ml) and Penicillin G (15 mg) in 70% Ethanol (10 ml). The effluent of the liquefying Melissa compartment, containing fibrous material, can be used in the additional aerobic reactor together with the cultivated organisms. The final product is recycled to the anaerobic reactor together with new feacal material. The survival of introduced species in the second "slow compartment" may be monitored by means of PCR-techniques. Biodegradation efficiencies of lignin and cellulose can be used to evaluate the biodegradation of this specific components. Total carbon biodegradation efficiencies can be evaluated by measuring the CO_2 production.

4.2 Preliminary tests with commercial enzymes and faeces

Cellulose and xylan are two components which are difficult to biodegrade by the anaerobic bacteria. The pretreatment of faeces with enzymes can promote the biodegradability of those recalcitrant components. Therefore test can be set-up to investigate if pretreatment of the faeces has a possitive effect on the biodegradation of cellulose and xylan.

Different concentrations of a commercial enzyme (cellulase, xylanase) is incubated with a substrate (cellulase, xylan). This incubation is performed in the optimal conditions of the enzyme (cellulase: pH= 4.8, T= 30°C; xylanase: pH= 5.4, T= 30°C). After this incubation non diluted faecal material (5 ml) from the liquifying compartment in the MELISSA-loop is brought together with an anaerobic medium (20 ml) and the enzyme/substrate solution (35 ml). The headspaces of the bottles are flushed with a gas mixture containing 70% nitrogen and 30% carbondioxide prior to incubation at 50°C in order to obtain anaerobic conditions. All set-ups are preformed in triplicate. Based on the evolution of the pressure build up in the headspace, the gas production is measured. With these results and the analyses of FVA for each bottle, the biodegradation efficiencies can be evaluated.

5. Conclusions

Biodegradation of lignocellolytic material is investigated and reported by a number of authors. It was proven that high biodegradation rates can be achieved. Yet, biodegradation efficiencies are quite divers and not universal applicable due to the complex structure of fibrous material. The nature of fibrous components obstructs also the chemical determination of the fibrous material. Quantitative analyses of fibres relies on extraction and digestion procedures and gravimetric measurement. Semi-qualitative analyses are obtained by determining reducing sugars after acid digestion.

Cellulose is biodegradable by a wide range of organisms but the biodegradation efficiency is strongly dependent of the structure of the cellulose and the linkage with lignin and hemicellulose. It is known that in anaerobic conditions especially lignin is hardly biodegraded. In aerobic conditions, the bacterial group Actinomycetes and the White rot fungi

(Basidiomecytes) are capable to biodegrade lignocellolytic components by means of specific enzymes and their mycelium growth. This characteristics make this organism interesting to introduce in the Melissa-cycle, using an additional aerobic reactor, for further biodegradation of the resistant part of the organic material.

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