

Editorial Manager(tm) for Biodegradation
Manuscript Draft

Manuscript Number:

Title: Development of an enzymatic assay for the determination of cellulose bioavailability
in Municipal Solid Waste

Article Type: Original research article

Section/Category:

Keywords: biodegradability; biochemical methane potential assay; cellulosic compounds;
enzymatic hydrolysis test; municipal solid waste

Corresponding Author: Mr Christian Rodriguez University of Liège

First Author: Christian Rodriguez, MD

Order of Authors: Christian Rodriguez, MD; Serge Hilgsmann, Ir; Marc Ongena, Dr;
Robert Charlier, Pr; Philippe Thonart, Pr

Abstract:

TITLE

Development of an enzymatic assay for the determination of cellulose bioavailability in Municipal Solid Waste

AUTHORS

Christian Rodriguez¹, Serge Hiligsmann¹, Marc Ongena¹, Robert Charlier³ & Philippe Thonart^{1,2}

AFFILIATIONS AND ADDRESSES

¹ Walloon Center of Industrial Biology, Unit of Microbial Technology, University of Liège, B40, B-4000 Sart-Tilman, Belgium.

² Walloon Center of Industrial Biology, Unit of Bio-Industries, Faculty of Agricultural Sciences of Gembloux, Passage des Déportés, 2, B- 5030 Gembloux, Belgium.

³ Department of Géomac, University of Liège, B52/3, B-4000 Sart-Tilman, Belgium.

COMMUNATING AUTHORS

Rodriguez Christian

Walloon Center of Industrial Biology, Unit of Microbial Technology, University of Liège, B40, B-4000 Sart-Tilman, Belgium.

Phone : ++32.4.366.39.99

Fax : ++32.4.366.28.61

E-mail : ch.rodriguez@ulg.ac.be

KEYWORDS

biodegradation, cellulosic compounds, enzymatic hydrolysis test, biochemical methane potential assay, municipal solid waste

ABSTRACT

As there is a constant need to assess the biodegradation potential of refuse disposed of in landfills, we have developed a method to evaluate the biodegradability of cellulosic compounds (cellulose and hemicellulose) in municipal solid waste. This test is based on the quantification of monosaccharids released after the hydrolysis of solid waste samples with an optimised enzyme preparation containing commercially available cellulases and hemicellulases. We show that the amounts of monosaccharids could be related to the biodegradability of the cellulosic material contained in the samples. This enzymatic cellulose degradation test was assayed on 26 samples originating from two Belgian landfills and collected at different depths. As results correlated well with those obtained with a classical biochemical methane potential assay, this new and rapid test is sufficiently reliable to evaluate cellulose bioavailability in waste samples.

ABREVIATIONS

INTRODUCTION

Municipal solid waste (MSW) has been disposed of in landfills for several decades. The organic matter contained in the landfill body is degraded microbiologically generating leachate and biogas that have to be managed for several years. There is thus a constant need to assess the biodegradability of buried MSW in order to evaluate the efficiency of different MSW pretreatments, to predict the duration of the aftercare period or to estimate the remaining potential for landfill gas production.

The gas potential can be indirectly determined via stoichiometric and empirical equations from the determination of total organic carbon (TOC), chemical oxygen demand (COD) and other specific parameters such as cellulose and lignin contents (Chandler et al, 1980; Parkin, G.F. & Owen, W.F., 1986; Metcalf & Eddy Inc., 1991; Wang et al., 1997). It is also possible to measure the calorific value (H_0) describing the potential amount of energy that will be gained in an incineration process. Alternatively, powerful analytical methods such as NMR and FT-IR spectroscopy, have been developed to monitor the changes in the chemical structure of MSW during composting (Pichler et al., 2000 and Smidt et al., 2002). Some biological tests based on aerobic and anaerobic assays have also been developed to evaluate the biodegradability of MSW and the gas generating potential. At the same time, several workers have estimated the biodegradability of solid waste components by the use of a biochemical methane potential (BMP) assay (Shelton & Tiedje, 1984; Bogner, 1990; Wang et al., 1994; Stinson & Ham, 1995; Eleazer et al., 1997) or by an incubation test (Binner et al., 1999). Both assays are based on the measure of methane gas produced by a methanogenic biomass degrading the organic matter in anaerobic conditions. Other tests evaluate the biodegradability of organic polymers and residual wastes by measuring the

oxygen consumed or the carbon dioxide produced during a respiration test (Pagga et al., 1995; Binner et al., 1999).

Whilst different methods offer certain advantages, they also suffer from certain limitations. For instance, chemical parameters such as COD and TOC do not take into account the biodegradable fraction of the organic matter. Spectroscopic methods require sophisticated equipment and are limited to the study of chemical transformations. Anaerobic tests need to be run for several months and respiration tests simulate aerobic conditions that do not prevail into the landfill.

The organic fraction of MSW is made up of 30-50 % of cellulosic substances that can undergo biological degradation (Rees, 1980; Barlaz et al., 1989; Eleazer et al., 1997). Cellulose and hemicellulose are therefore the most significant carbon source for methanogenesis in landfills as their degradation contributes to 90% of the total methane produced (Barlaz et al., 1989). However, the biodegradation of cellulosic substrates, such as paper, cardboard, wood and textile, is very slow with a half-life of about 15 years (Gendebien et al., 1992) and therefore represents one of the limiting steps of the biological processes occurring in MSW landfills.

Our study focused on the first stage of the bioconversion process, *i.e.* the enzymatic hydrolysis step. In this work, a new test allowing a reliable and rapid evaluation of the enzymatic cellulose bioavailability was developed. This test was based on enzymatic hydrolysis of residual cellulosic material to quantify the biodegradability with subsequent measurement of the quantity of sugars liberated. This enzymatic cellulose degradation test (ECD) has been performed on refuse samples originating from various layers of two different landfills and results were compared with those obtained from BMP assays realised in parallel.

MATERIAL AND METHODS

Sample preparation

Waste refuses were collected from boreholes (up to 35 m-depth) made in two Belgian landfills L1 and L2. Waste was extracted from a borehole and separated into samples corresponding to 1 m intervals. Large glass pieces, stones, plastics and metal pieces were removed manually while the remaining refuse materials were shredded with a cutting mill to a particle size of ≤ 5 mm and homogenised. Samples containing 3 to 35 % cellulosic material were then dried at 105°C for 24 h.

Chemical analysis

Cellulosic materials were analysed according to a HPLC method adapted from Pettersen & Schwandt (1991). 300 mg of each MSW sample was hydrolysed with 3 ml of 72 % H_2SO_4 for 1 h at 30 °C. The samples were then diluted to 2.5 % H_2SO_4 with distilled water and autoclaved at 120 °C for 1h. Samples were run in triplicate and D (+) Fucose (Fluka, Buchs, Switzerland) was used as standard to correct for further hydrolysis due to the autoclave operation. Samples were analysed by HPLC on an Agilent 1100 series apparatus (Agilent Technologies, Massy, France) equipped with a refractometric detector. Sugars were separated on a C-610-H ion exchange column (300 mm x 7,8 mm, Supelco, Bellefonte, PA.) and quantified using standards. All samples were filtered through 0,2 μm Minisart Syringe filter (Vivascience, Hannover, Germany) prior to analysis.

Lignin was determined gravimetrically following extraction with triethylene glycol as described by Edwards, 1972 and after a clean-up procedure of the waste material with a modified neutral detergent fibre (NDF) pre-treatment (Rowland and Roberts, 1999).

Enzymatic hydrolysis test

Enzymes

The enzymes used for the hydrolysis test were all purchased from Novo Nordisk (Bagsvaerd, Denmark). Viscozyme L[®] and Celluclast 1.5L[®] are liquid cellulolytic preparations and Celluzyme[®] is a solid cellulolytic preparation.

Celluzyme solutions were prepared by dissolving the commercial product in 0.1 N phosphate buffer at pH 5.5 to which 0.05 % NaNO₃ was added to prevent microbial growth. The solutions were then filtered on a GF/C membrane (Whatman, Maidstone, England). Celluclast 1.5L and Viscozyme L were dialysed overnight in the same buffer using nitrocellulose membranes with a cut-off of 10 kD (Sigma-Aldrich, S^t Louis, USA). One litre of the working enzymes mixture was obtained by adding 500 ml of Celluzyme 20 g/l, 100 ml of dialysed Viscozyme L and 50 ml of dialysed Celluclast 1.5L to 350 ml of 0.1 N phosphate buffer-0.05 % NaNO₃ at pH 5.5.

Determination of enzyme activities

The filter paper assay (FPase activity) was used for cellulase activity determination (Mandels et al., 1976). Endoglucanase (CMCase) and β -glucosidase activities were measured after incubating 200 μ l of enzymatic solution with respectively 1500 μ l of

carboxymethylcellulose 1 % and 1500 µl cellobiose 1 % (adapted from Miller et al., 1960 & Gordon and Phillips, 1989), both prepared in the same buffer as mentioned above before being heated for 2 min at 100°C to stop the reaction. Hemicellulase (xylanase) activity was determined by using oats spelt xylan (Sigma-Aldrich, S^t Louis, USA) following the procedure for filter paper assay.

All activities were calculated after 1 hour at pH 5.5 and 40 °C. In all cases, one enzyme unit was defined as the quantity of micromoles of monosaccharid liberated per minute. According to the technical data given for each enzyme, pH and temperature values were fixed so as they cover the range allowing an optimal activity.

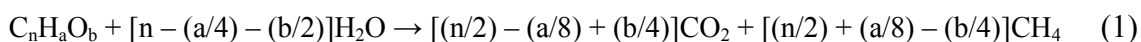
The cellulase and hemicellulase activities of Celluzyme, Celluclast 1.5L and Viscozyme L were first tested in order to determine the best compromise to use them in a mixture. Celluzyme activities were tested at 5, 10 and 20 g/l. Celluclast and Viscozyme activities were tested after being respectively diluted 20, 50, 100 times for the Celluclast and 10, 50, 100 times for the Viscozyme. For each dilution, controls were made to measure the background of sugars already present in Novo Nordisk enzymatic preparations.

Kinetic of enzymatic hydrolysis

Cellulase and hemicellulase-mediated hydrolysis were performed either with each enzyme (Celluzyme, Celluclast 1.5L and Viscozyme L) preparation or with a mixture of all three. For hydrolysis, 1000 mg of sample were mixed with 30 ml of an enzymatic solution for 40 hours at 40 °C. The biodegradability of refuse samples is evaluated by the mass of monosaccharids liberated reported to the total mass of sample hydrolysed.

Biochemical Methane Potential (BMP) assay

The BMP assay and the volumes of methane produced were determined following the procedure described by Wang et al. (1994). The concentrations of methane and carbon dioxide in the biogas produced in a BMP assay were measured on a gas chromatograph (Hewlett Packard 5890 series II) equipped with a thermal conductivity detector (TCD) using a GasPro GSC column (30 m x 0,32 mm,) (Alltech, Deerfield, USA) coupled to a CP-Carboplot P7 column (27,5 m x 0,53 mm, Varian, Middelburg, The Netherlands). Helium N45 (Air Liquide, Liège, Belgium) was used as carrier and reference gas. Calibration was performed using gas mixtures standards purchased by Air liquide (Liège, Belgium). Equation 1 (Parkin and Owen, 1986) was used to calculate the theoretical methane potential of monosaccharids when converted to methane.



RESULTS

Cellulolytic and hemicellulolytic activities of enzymes used

Cellulase (FPase) and xylanase activities of the different commercial products (Celluzyme, Celluclast and Viscozyme) and the content of sugars already present in these preparations (background) were measured at various concentrations (table 1). All three original enzyme solutions had both xylanolytic and cellulolytic activities. These results enabled the determination of the best compromise between a high enzymatic activity and a low background, *i.e.* a mixture containing Viscozyme and Celluclast

diluted 10 and 20 times respectively and 10 g/l of Celluzyme. The resulting activities of the mixture show a FPase activity of 350 mIU/ml and a xylanase activity of 420 mIU/ml. The FPase and xylanase activities measured for the enzymatic mixture were close to the sum of each enzyme activity. Moreover, specific CMCase and cellobiase assays made sure that this mixture had endoglucanase (30 mUI/ml) and β -glucosidase (540 mUI/ml) activities. A lack of β -glucosidase activity would lead for example to an accumulation of cellobiose that is known for its feedback effect on cellulases. An efficient β -glucosidase activity is also essential in order to degrade cellulose completely to monomeric sugars that will be quantified by HPLC.

Enzymatic hydrolysis of cellulosic substrates

In a next step, enzymatic hydrolysis was performed on cellulosic (Whatman n°1 paper), and hemicellulosic (xylan from oat spelts) substrates in order to investigate the time needed to reach the end of the kinetic phase and to determine the concentrations of glucose and xylose associated with the decrease of the enzymatic activity. The hydrolysis associated with the degradation of 500 mg of these substrates was followed for 30 hours. Each enzyme and the enzyme mixture was tested in triplicate (figure 1). For both substrates, the rate of hydrolysis was higher during the first five hours of incubation and decreased after 20 hours (beginning of the stationary phase). With respectively 80 and 50 % of cellulose and xylan hydrolysed after 30 hours, the mixture of enzymes increased significantly the hydrolysis yield in comparison with each enzyme tested alone. This degradation of cellulose and xylane was associated with an accumulation of glucose and xylose that reached respectively 15 and 10 g/l in the media. This gives an indication of the concentration of monosaccharids that could be

obtained when other cellulosic substrates are degraded without being interpreted as a limiting enzymatic activity if the concentrations reached are lower.

Enzymatic hydrolysis was also performed on spruce wood (figure 2), containing 51 % cellulosic materials and 29 % of lignin. Wood was tested because their cellulosic compounds are closely linked to lignin, limiting therefore the bioavailability of these polysaccharids. Results showed a lower percentage of hydrolysis compared to those obtained with substrates such as pure cellulose and xylan (figure 1). The level of degradation induced by the mixture of cellulases was similar to that observed with celluclast as only 0.6 g/l of monosaccharids was released into the medium. This relatively low yield of hydrolysis led to the question of whether enzyme inhibition or bioavailability was limiting cellulose/hemicellulose conversion to glucose/xylose. To address this, a cellulose spike (100 mg of Whatman n°1 filter paper) was added to the enzymatic medium for 14 hours after 30 hours of incubation. The medium spike recovery was 78 % of the glucose expected from filter paper addition. These data suggest that there was not an environmental condition within the enzymatic cellulose degradation (ECD) test that limited cellulose conversion to glucose, but rather the bioavailability of the cellulose.

Comparison of ECD and BMP assays on MSW samples

The BMP assay, which involves an anaerobic process close to the one taking place in a landfill, was compared to the ECD test. Both tests were performed on waste samples collected from various layers of two different MSW landfills (L1 and L2). Therefore, the selected samples had distinct chemical compositions (from 3 to 35 % of cellulosic material) and different disposal times (from several months to more than 20 years). The

monosaccharids or methane respectively released were reported to the mass of the sample in order to describe the potential of biodegradation of cellulosic substances in MSW samples.

The Figure 3 shows the correlation between the total specific amount of sugars liberated after 48 hours of enzymatic hydrolysis and the total specific volume of methane produced after 100 days of anaerobic degradation. The two measures appear to be significantly correlated (calculated with a Student test, $P = 0.05$) both for samples from L1 ($r^2 = 0.87$) and L2 ($r^2 = 0.65$). However, the regression lines have different slopes although there is still a globally significant correlation ($r^2 = 0.46$) when all the 26 samples from L1 and L2 are considered together. On the other hand, the volumes of methane experimentally measured for samples L1 are close to those theoretically produced if all the sugars released during the ECD test were converted to methane (figure 3). This is not the case for samples L2 where experimental methane potential is higher than the theoretical methane potential of the sugars released by the ECD test suggesting that MSW samples were more completely degraded by the anaerobic biomass.

Assessment of the enzymatic hydrolysis

Further experiments have been carried out to validate the enzymatic test and particularly to achieve a complete hydrolysis of the cellulose bioavailable. The samples submitted to the enzymatic hydrolysis were dried at 50 °C to constant weight and then submitted to a second, and in the same way, to a third hydrolysis. The figure 4 shows the average proportion of each hydrolysis compared to the total percentage of cellulose hydrolysed. The first hydrolysis degraded on 83 % of the total amount of the cellulose bioavailable

after three hydrolysis. The second and the third hydrolysis degraded respectively 11 and 6 %. In the case of the samples coming from L1, the correlation coefficient between the total specific amount of monosaccharids liberated by the enzymatic test and the total specific volume of methane produced by the BMP test rises from 0.87 to 0.91 after the second hydrolysis and to 0.92 after the third one. However, this correlation coefficient decreases from 0.69 to 0.64 and to 0.47 for samples coming from L2. Anyway, the low concentrations measured after the first hydrolysis in most of the samples suggest that one hydrolysis is sufficient enough to calibrate the test with a BMP assay.

DISCUSSION

The results presented in this paper show that the ECD test describes as well as the anaerobic BMP assay the degradation potential of MSW samples collected at various depths in two different landfills. Other works also compared the results of anaerobic tests to other assays based on respiration activity or volatile solids measurements (VS), Binner et al. (1999) showed a good relationship between results from a 7 days respiration assay and an anaerobic assay running over 90 days when both were applied to 23 MSW samples coming from different mechanical biological pre-treatment plants. They also showed that the respiration activity was related to the mass lost by the samples after ignition at 1000°C (Ignition Loss) but the correlation was only significant for the samples coming from the same treatment plant. By comparing different stability criteria for mechanical biological pretreated waste, Cossu et al. (2001) also showed a relationship between a respiration activity and an anaerobic fermentation test but only 6 samples were considered in this case.

However, the biodegradation potential evaluated by respiration assays or by some chemical analysis (TOC and VS) do not take into account the non biodegradability of some organic compounds under the anaerobic conditions taking place in landfills. For example, lignin that is intimately associated with cellulose in woody tissues and plants, is only slowly degradable under anaerobic conditions (Young and Frazer, 1987; Colberg, 1988). Therefore, its resistance is thought to delay strongly the biodegradation of the cellulosic material (Crawford, 1981) due to a lack of cellulose availability. On the other hand, the main disadvantage of anaerobic tests, such as a BMP assay, is that they must be carried out over a very long period (more than 100 days). In this context, the ECD test we report here is more appropriate as it assesses the fraction of cellulose that is readily available without changes of the lignin properties. Results from ECD test and BMP assay applied to 26 samples from two Belgian landfills showed a significant correlation. However, the regression slopes between ECD and BMP results were quite different in the two considered landfills. The lower slope of the regression line L2 (figure 3) implies that MSW samples were more completely degraded by the anaerobic biomass, suggesting that cellulose was more available for the anaerobic microflora than for the enzymatic mixture even if this mixture was active enough to degrade all the cellulose contained in the samples. The presence of other carbon sources (proteins, lipids) as substrates for the anaerobic microflora in the BMP assay or as a barrier limiting cellulose bioavailability for enzymes in the ECD test might also explain the variations observed between L1 and L2 samples. However, protein and lipids respective contents are usually not higher than 5-6 % in fresh MSW (Rees, 1980; Barlaz et al., 1989) and 5-8 % of the TOC in old waste Bäumler et al. (2001). Moreover, Gendebien et al. (1992) considered that food waste, that is mainly composed of proteins and lipids, which have a relatively short half-life time of 1 year.

Nevertheless, our results show that the ECD test combined with the BMP assay could highlight a different trend between samples coming from two different landfills. Such a differential bioconversion behaviour of cellulosic substances to methane reinforces the need for parameters evaluating the biodegradation potential instead of, or in combination with, other chemical measurements like TOC, VS, COD...

In fact, the study of MSW with combined tests gives a good idea of the methane potential still expected from the mass of enzymatically degraded (hemi)cellulose. Moreover, limit values can be recommended, as suggested by Binner et al. (1999), in order to define MSW with a low biodegradation potential. For example, assuming that gas generating potential of fresh MSW ranges between 100 and 200 Nl/kg MSW (Barlaz et al., 1990; Pacey, 1990; Gendebien et al., 1992, Binner et al., 1999), a limit value of 10 % of this potential (10-20 Nl/kg) could be considered as acceptable to classify waste samples as sample with a low methane potential. The correlating values with the ECD when the 26 samples of L1 and L2 are considered together ranges between 10 and 20 g of monosaccharids / kg of waste.

CONCLUSIONS

In this paper, a new and rapid enzymatic test using a mixture of cellulases/hemicellulases has been compared to a classic 100 days-BMP assay in order to assess the cellulose degradation of MSW. Both methods have been performed on two sets of MSW samples under suitable conditions for biodegradation *i.e.* no limiting moisture content, optimal pH and temperature. The results show a good correlation between the two assays. As it allows a large set of trials with reduced incubation time, this enzymatic test is a promising tool to study the biodegradation potential of cellulosic

material in MSW samples. Moreover, it simulates the microbial degradation of cellulose in the presence of the lignin barrier using high activities of (hemi)cellulolytic enzymes. It may thus assess rapidly the methane potential of waste refuses and may point out different behaviours of bioconversion when combined with methanisation tests.

Acknowledgement

The study was financed by funds *Actions de Recherche Concertées* from the *Direction générale de l'Enseignement non obligatoire et de la Recherche scientifique- Direction de la Recherche scientifique* - French talking community of Belgium.

References

- Barlaz MA, Ham RK & Schaefer DM (1989) Mass balance analysis of anaerobically decomposed refuse. *J. Env. Eng.* 115 (6): 1088-1089
- Barlaz MA, Ham RK & Schaefer DM (1990) Methane production from municipal refuse: a review of enhancement techniques and microbial dynamics. *Crit. Rev. Environ. Control* 19(6): 557-584
- Bäumler R, Lindel H, Knicker H & Kögel-Knabner I (2001) Stability of organic matter in an old landfill site – a case study in Northern Bavaria (Germany). In: T.H. Christensen, R. Cossu and R. Stegmann (Ed) *Proceedings Sardinia 01, Eighth International Waste Management and Landfill Symposium, Santa Margherita di Pula, Cagliari, 1-5 Oct. 2001, Vol 4 (457-464)*, Cisa, EuroWaste Srl, Padova
- Binner E & Zach A (1999) Biological reactivity of residual wastes and dependence on the duration of pretreatment. *Waste Manage. Res.* 17: 543-554

368 Bogner JE (1990) Controlled study of Landfill biodegradation rates using modified
 369 BMP assays. *Waste Manage. Res.* 8: 329-352
 370 Chandler JA, Jewell WJ, Gossett JM, Van Soest PJ & Robertson JB (1980) Predicting
 371 Methane Fermentation Biodegradability. *Biotechnol. Bioeng. Symp.* 10: 93-107
 372 Colberg PJ (1988) Anaerobic microbial degradation of cellulose, lignin, oligolignols
 373 and nonaromatic lignin derivatives. In : Zehnder AJB (Ed) *Biology of Anaerobic*
 374 *Microorganisms* (pp 333-372). Wiley-Liss, New-York
 375 Cossu R, Raga R & Vascellari V (2001) Comparison of different stability criteria for
 376 MBP waste in view of landfilling. In: T.H. Christensen, R. Cossu and R. Stegmann (Ed)
 377 *Proceedings Sardinia 99, Seventh International Waste Management and Landfill*
 378 *Symposium, Santa Margherita di Pula, Cagliari, 4-8 Oct. 1999, Vol 1 (473-478), Cisa,*
 379 *EuroWaste Srl, Padova*
 380 Crawford R L (1981) *Lignin Biodegradation and Transformation*. Wiley-Interscience,
 381 New-York
 382 Edwards CS (1973) Determination of lignin and cellulose in forages by extraction with
 383 triethylene glycol. *J. Sci. Food Agr* 24: 381-388
 384 Eleazer W E, Odle WS, Wang YS & MA Barlaz (1997) Biodegradability of municipal
 385 solid waste components in laboratory-scale landfills. *Environ. Sci. Technol.* 31: 911-
 386 917
 387 Gendebien A, Pauwels M, Constant M, Ledrut-Damanet M-J, Nyns EJ, Willumsen H-C,
 388 Butson J, Fabry R & Ferrero G-L (1992) The process of methanogenesis in landfills. In:
 389 *Landfill gas. From environment to energy* (pp121-140). Commission of the European
 390 Communities, Luxemburg

391 Gordon, GLR & Phillips MW (1989) Degradation and utilisation of cellulose and straw
 392 by three different anaerobic fungi from the ovine rumen. *Appl. Environ. Microb.* 55 (7):
 393 1703-1710
 394 Mandels M, Andreotti, R & Roche C (1976) Measurement of saccharifying cellulase.
 395 *Biotechnol. Bioeng. Symp.* 5: 21-33
 396 Metcalf & Eddy Inc. (1991). *Wastewater Engineering: Treatment, Disposal and Reuse*,
 397 3rd Edition, McGraw Hill Inc, New York
 398 Miller GL, Blum RWE, Glennon WE & Burton AL (1960) Measurement of
 399 carboxymethyl cellulase activity. *Anal. Biochem.* 1: 127-132
 400 Pagga U, Beimborn DB, Boelens J & De Wilde B (1995) Determination of the aerobic
 401 biodegradability of polymeric material in a laboratory controlled composting test.
 402 *Chemosphere.* 31(n°11/12): 4475-4487
 403 Pacey J G (1990) *Sanitary landfilling: process, technology and environmental impact*.
 404 Academic press, London
 405 Parkin GF and Owen WF (1986) Fundamentals of anaerobic digestion of wastewater
 406 sludges. *J. Environ. Eng. Div. ASCE* 112 (EE5): 867-920
 407 Pettersen, RC and Schwandt, VH (1991) Wood sugar analysis by anion
 408 chromatography. *J. Wood Chem. Technol.* 11: 495-501
 409 Pichler M, Knicker H & Kögel-Knabner I. Changes in the chemical structure of
 410 municipal solid waste during composting as studied by solid-state dipolar dephasing and
 411 PSRE ¹³C NMR and solid-state ¹⁵N NMR spectroscopy. *Environ. Sci. Technol.* 34 (18):
 412 4034-4038
 413 Rees, JF (1980). The fate of Carbon Compounds in the Landfill Disposal of organic
 414 Matter. *J. Chem. Technol. Biotechnol.* 30: 161-175

415 Rowland AP & JD Roberts (1999) Evaluation of lignin and lignin-nitrogen fractionation
 416 following alternative detergent fiber pre-treatment methods. *Commun. Soil Sci. Plant*
 417 *Anal.* 30 (1&2): 279-292
 418 Shelton DR & Tiedje JM (1984) General method for determining anaerobic
 419 biodegradation potential. *Appl. Environ. Microb.* 47 (4): 850-857
 420 Smidt E, Lechner P, Schwanninger M, Haberhauer G and Gerzabek MH (2002).
 421 Characterization of waste organic matter by FT-IR spectroscopy – Application in waste
 422 science. *Appl. Spectrosc.* 56: 1170-1175
 423 Stinson, JA & Ham, RK (1995) Effect of lignin on the anaerobic decomposition of
 424 cellulose as determined through the use of a biochemical methane potential method.
 425 *Environ. Sci. Technol.* 29, 2305-2310
 426 Wang YS, Byrd CS & Barlaz MA (1994) Anaerobic biodegradability of cellulose and
 427 hemicellulose in excavated refuse samples using biochemical methane assay. *Journal of*
 428 *Industrial Microbiology.* 13, 147-153
 429 Wang YS, Odle WS, William E, Barlaz MA (1997) Methane potential of food waste and
 430 anaerobic toxicity of leachate produced during food waste decomposition. *Waste*
 431 *Manage. Res.* 15, 149-167
 432 Young LY & Frazer AC (1987). The fate of lignin and lignin-derived compounds in
 433 anaerobic environments. *Geomicrobiol. j.* 5: 261-293
 434

Figure Legend

Fig.1 Cellulose or hemicellulose hydrolysed when Whatman n°1 paper (A) and Xylan from oats spelt (B) are degraded for 30 h with Viscozyme (10 fold diluted) (▲), with Celluzyme (10 g/l) (■), with Celluclast (20 fold diluted) (◆) and with the enzymatic mixture (●). Concentrations of glucose (A) or xylose (B) released during the degradation of Whatman n°1 paper and Xylan with the enzymatic mixture.

Fig.2 Cellulosic substances (cellulose and hemicellulose) hydrolysed when spruce wood are degraded for 30 h with Viscozyme (10 fold diluted) (▲), with Celluzyme (10 g/l) (■), with Celluclast (20 fold diluted) (◆) and with the enzymatic mixture(●). Concentrations of monosaccharids released during the degradation of the spruce wood with the enzymatic mixture.

Fig.3 Relationship between the total specific amount of monosaccharids (and the corresponding methane potential) liberated by the enzymatic test after 48 h and the total specific volume of methane produced by a 100 days-BMP test. The 26 samples tested are originating from different layers of two different Belgian landfills called L1 (◆) and L2 (□).

Fig. 4 Proportion of the total percentage of cellulose and hemicellulose hydrolysed after each of the 3 hydrolysis. The 26 samples tested are originating from different layers of landfills L1 and L2.

Table 1 FPase and xylanase activities of Celluzyme, Viscozyme, Celluclast and enzymatic mixture measured at different concentrations^a or dilutions^b of the commercial products. Background of sugars measured in the different enzymatic preparations

Enzymes	Concentration (g l ⁻¹) dilution factor	FPase activity (mUI ml ⁻¹)	Xylanase activity (mUI ml ⁻¹)	Background (g l ⁻¹)
Celluzyme ^a	2.5	39	nd	0.073
	5	60	nd	0.111
	10	85	200	0.15
	20	121	nd	0.319
Celluclast ^b	20	176	135	0
	50	109	84	0
	100	68	53	0
Viscozyme ^b	10	62	100	0,23
	50	nd	35	0
	100	nd	32	0
Enzymatic mixture ^c	-	350	420	nd

nd: non determined

^c For 1 litre : 500 ml of Celluzyme 20 g/l, 100 ml of dialysed Viscozyme L and 50 ml of dialysed Celluclast 1.5 L and 350 ml of 0.1 N phosphate buffer-0,05 % NaN₃ at pH 5.5.

Figure 1

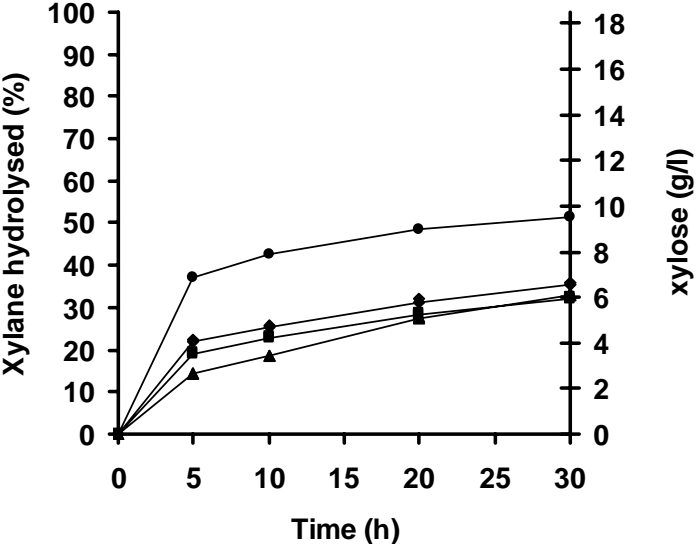
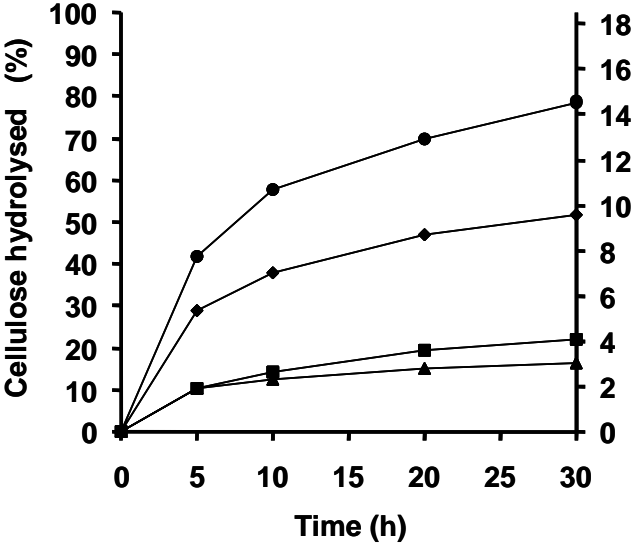
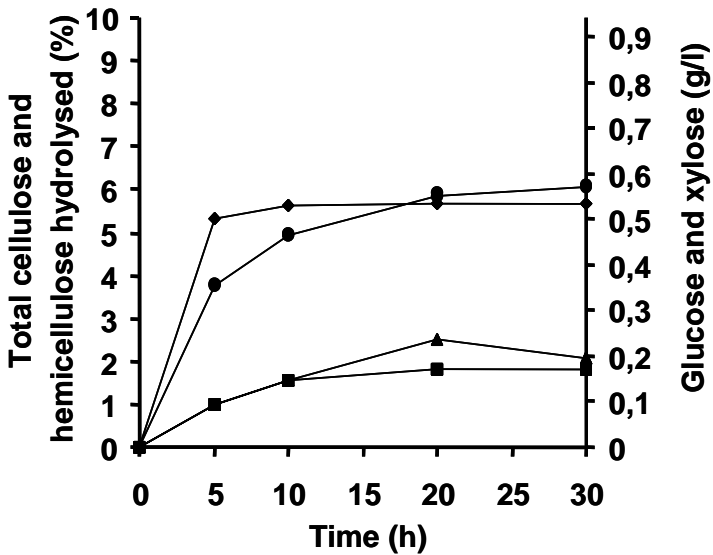
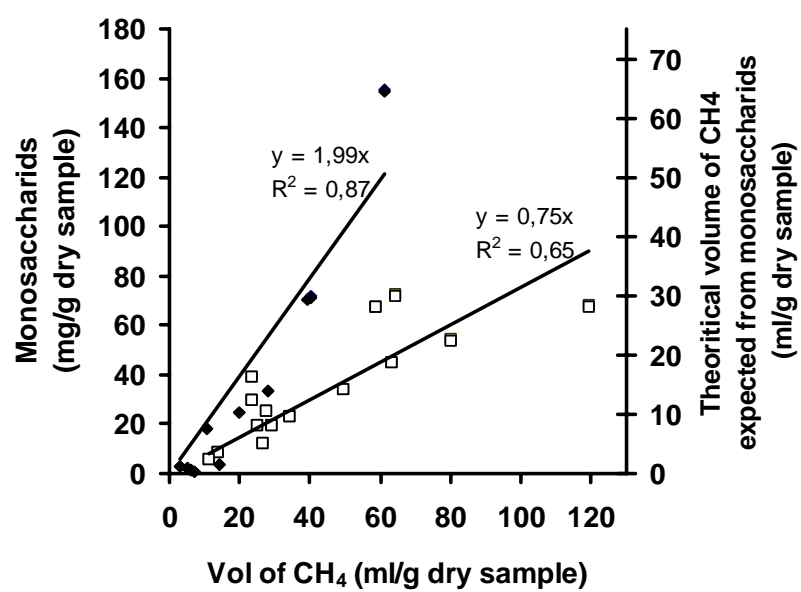


Figure 2



475 **Figure 3**



476

477

478

479

480

Figure 4

