Biodegradation **15:** 173–183, 2004.

Advanced anaerobic bioconversion of lignocellulosic waste for bioregenerative life support following thermal water treatment and biodegradation by *Fibrobacter succinogenes*

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Accepted 17 December 2003

Key words: biogas, biosolids, carbon cycling, food waste, hydrothermolysis

Abstract

The feasibility of nearly-complete conversion of lignocellulosic waste (70% food crops, 20% faecal matter and 10% green algae) into biogas was investigated in the context of a life support project. The treatment comprised a series of processes, i.e., a mesophilic laboratory scale CSTR (continuously stirred tank reactor), an upflow biofilm reactor, a fiber liquefaction reactor employing the rumen bacterium *Fibrobacter succinogenes* and a hydrothermolysis system in near-critical water. By the one-stage CSTR, a biogas yield of 75% with a specific biogas production of 0.37 l biogas g^{-1} VSS (volatile suspended solids) added at a RT (hydraulic retention time) of 20–25 d was obtained. Biogas yields could not be increased considerably at higher RT, indicating the depletion of readily available substrate after 25 d. The solids present in the CSTR-effluent were subsequently treated in two ways. Hydrothermal treatment (T $\sim$ 310–350 °C, p $\sim$ 240 bar) resulted in effective carbon liquefaction (50–60% without and 83% with carbon dioxide saturation) and complete sanitation of the residue. Application of the cellulolytic *Fibrobacter succinogenes* converted remaining cellulose contained in the CSTR-effluent into acetate and propionate mainly. Subsequent anaerobic digestion of the hydrothermolysis and the *Fibrobacter* hydrolysates allowed conversion of 48–60% and 30%, respectively. Thus, the total process yielded biogas corresponding with conversions up to 90% of the original organic matter. It appears that particularly mesophilic digestion in conjunction with hydrothermolysis at near-critical conditions offers interesting features for (nearly) complete and hygienic carbon and energy recovery from human waste in a bioregenerative life support context.

Introduction

In bioregenerative LSS (life support systems), lignocellulosic crop residues and biosolids (e.g., faeces) represent an important source of biochemical energy both for energy recovery and for the subsequent production of foods in space (Kohlmann et al. 1995; Strayer & Atkinson 1997; Fulget et al. 1999; Kudenko et al. 2000). Microbial cellulose utilization in natural environments is responsible for one of the largest material flows in the biosphere and is of great interest in relation to carbon cycling at global and local scales (e.g., life support) (Lynd et al. 2002).

On earth, anaerobic digestion of various organic wastes is a well-established technology in which part of the energy can be recovered in the form of biogas (Schober et al. 1999; Zhang & Zhang 1999; Stroot et al. 2001; Liu et al. 2002). The anaerobic decomposition process can be divided in four steps of which the first step, the hydrolysis of particulate biopolymers

(e.g., cellulose, hemicellulose), is considered as the rate-limiting step for the overall process (Schieder et al. 2000; Sanders et al. 2000). Because certain cellulosic polymers are shielded by lignin in a solid and water-insoluble structure, these polymers have little bioavailability for many fermenting microorganisms (Ahring et al. 1999; Liu et al. 2002).

Various (thermo) chemical and biochemical hydrolysis methods that split plant biopolymers into water-soluble and biodegradable short-chain compounds have been the subject of investigation in recent years (Delgenes et al. 2000; Schieder et al. 2000; Kim & Hong 2001; Liu et al. 2002). By employing pretreatments, biogas yields and conversion rates from organic waste can be enhanced and retention times can be lowered, allowing for more compact digester systems.

Thermal hydrolysis technologies have been explored as pre-and post treatment for the anaerobic digestion of lignocellulose. These technologies can be divided into wet oxidation (Schmidt et al. 1998), steam explosion (Saddler et al. 1993) and hydrothermolysis (Schieder et al. 2000). During treatment, lignocellulose is (partially) degraded into smaller fragments (cellulose, hemicellulose, lignin and sugar derivatives) by the action of hot water or steam under moderate pressures (e.g., 5–50 bar) and temperatures (180–325 °C), either in the absence or presence of a catalyst (e.g., pressurised oxygen) (Schmidt & Thomsen 1998; Lendormi et al. 2001; Bonmati et al. 2001). The thermal pre-hydrolysis step also offers other advantages such as complete sanitation of the waste and a decrease of the methane reactor volume (Schieder et al. 2000).

In biochemical hydrolysis processes, enzymes (e.g., cellulases) or (metabolically engineered) fermenting microorganisms are used to convert cellulosic compounds into monomeric sugars and/or organic acids at ambient temperatures and pressures. In this respect, ruminant cellulolytic bacteria are able to digest cellulose and produce organic acids such as succinate and acetate at high rates (Fields et al. 2000). The bacterium *Fibrobacter succinogenes* is widely considered as one of the most active and most important cellulose-digesting anaerobic bacteria in the rumen (Martin & Martin 1998). The objective of the present study was to determine the anaerobic digestion efficiency of a dilute organic substrate (2% ± 0.2 dry mass) composed out of food crops, faeces and green algae by means of anaerobic digestion completed by hydrothermolysis and cellulolytic digestion by *Fibrobacter succinogenes*. The study furthermore explores the potential of digester residue liquefied by a tubular near-critical (Tcrit = 374 °C, pkrit = 221 bar) high temperature/high pressure reactor. The carbon liquefaction power and fiber degradation of the hydrothermolysis and *Fibrobacter succinogenes* digestion was evaluated by a series of batch anaerobic digestion tests on the residual solids derived from primary CSTR anaerobic digestion. The overall biogas yield for anaerobic digestion in combination with hydrothermolysis and *Fibrobacter succinogenes* digestion was determined and the applicability for of the system for life support was evaluated.

Materials and methods

Substrate composition and preparation

The substrate was composed in such a way that it resembled a concentrated organic waste stream produced by humans in a LSS (Fulget et al. 1999). On DM (dry mass) basis the organic waste consisted of 70% crop residues (1/3 chopped wheat straw, 1/3 green cabbage, 1/3 soya waste), 10% of green algae (*Spirulina platensis*) and 20% of faecal matter. All components were suspended in tap water to obtain a final DM concentration of 2–3%. The suspension was stored at 4 °C.

Prior to anaerobic digestion, all substrate components except wheat straw were ground under wet conditions with a conventional kitchen grinder to obtain millimetre-sized particles. Wheat straw was ground in dry state with a rotary cutter that yielded straw particles in the millimetre range (1–3 mm). The characteristics of the individual substrate compounds are given in Table 1. The total substrate suspension (2% DM) had the following properties: COD = 21 g l^{-1} (chemical oxygen demand), TAN = 0.41 g l^{-1} (total ammonia nitrogen), Kj-N (Kjeldahl-nitrogen) = 1.4 g l^{-1}, VSS = 24 g l^{-1}, ash-content = 4.4 g l^{-1}, total fibers = 35%, (%) cellulose = 21%, (%) hemicellulose = 10% and (%) lignin = 4%.

After anaerobic digestion, the CSTR effluent solids were separated by centrifugation of the CSTR effluent at 7000 g for 15 min. Subsequently, the solids were recovered by decantation and were dried for minimum 24 h. These solids were than further treated in two ways (Figure 1). In process sequence 1, the CSTR solids were treated either by hydrothermolysis or *Fibrobacter* fermentation followed by a second sep-

Table 1. Composition of the compounds of the organic substrate in terms of DM (dry matter), COD (chemical oxygen demand), TC (total carbon) and TN (total nitrogen)

	Mass-% [gDM gDM^{-1}]	DM-content [gDM g^{-1}]	COD [gCOD gDM^{-1}]	TC [gC gDM^{-1}]	TN [gN gDM^{-1}]
Straw	0.23	0.93	1.30	0.39	0.0087
Soya	0.23	0.88	1.21	0.39	0.0166
Cabbage	0.23	0.08	1.26	0.37	0.045
Algae	0.1	0.95	1.49	0.42	0.1027
Faeces	0.2	0.2	n.d.	n.d.	n.d.

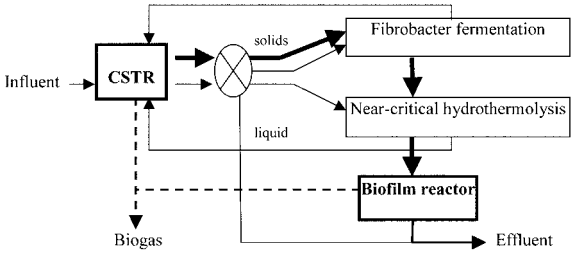

Figure 1. Conceptual scheme of the process sequences. The arrows indicate process sequence 1, the ***bold*** arrows indicate process sequence 2.

arate digestion for the sake of comparison of the biogas yields of the two treatments. In process sequence 2, the CSTR solids were first treated by *Fibrobacter* fermentation followed by hydrothermolysis and finally diverted to a second digestion (Figure 1).

The CSTR solids were subjected to additional size reduction steps prior to hydrothermolysis. Due to the small internal diameters of the high temperature/high pressure tubular reactor, the solid residues had to be ground to a sufficiently small particle diameter in order to prevent clogging of the apparatus. This was accomplished by means of a rotary cutter using two sieves in succession, having a mean mesh size of 1000 μm and 250 μm, respectively. The resulting material was additionally treated in a conventional coffee mill.

CSTR methanogenic reactor

A cylindrical 10 l PVC methanogenic reactor of CSTR-type was incubated at a constant temperature of 34 °C and was continuously shaken at 70 rpm (New Brunswick INNOVA, UK). The pH in the reactor was at a constant value of 7.3–7.4. The reactor contained 7.5 $\pm$ 1 l mixed liquour and was seeded with active methanogenic granular sludge from an anaerobic digester of a potato-processing firm (Primeur, Belgium). The produced biogas volume was measured daily and the biogas composition on a weekly basis.

Because of the particulate nature of the substrate, the reactor was fed manually and fed-batch wise. Prior to sampling, the content of the reactor was homogenized to prevent build-up of solids. The volumetric loading rate (B_v) of the mesophilic CSTR ranged from 1.5–2.5 g COD l^{-1} day^{-1} over a period of 12 months. The hydraulic retention time (RT) of the reactor was set at 20 d. Batch fermentation tests were performed with raw substrate at reaction times varying from less than 10 days to 65 days and at an initial concentration of 0–2.8 g l^{-1} COD to derive the influence of the initial concentration (g l^{-1} COD) and the RT (d).

High temperature/high pressure tubular reactor

The main building blocks of the tubular reactor are depicted in Figure 2. The high-pressure reaction unit is designed as a stainless steel tubular reactor (o.d.= 6.35 mm, i.d.= 3.05 mm) with a variable volume up to 100 ml and capable of withstanding operating pressures up to 300 bar and temperatures up to 450 °C.

Before entering the reaction unit the feed suspension is moderately preheated in an upstream coil, which has an inner volume of V_{Pre} = 38 ml. The reaction is started by mixing the feed suspension with a pure water stream (under nitrogen atmosphere), which is delivered into the system by a high-pressure membrane pump and heated to high temperatures in a second pre-heater. The pre-heater and the tubular reactor are electrically heated by means of heating jackets, which can be adjusted separately by a temperature control system (Horst HT-60 controller). In order to decrease the heat losses to the surroundings, the complete high temperature section of the apparatus is thermally insulated. The substrate suspension containing the particulate matter is fed into the sys-

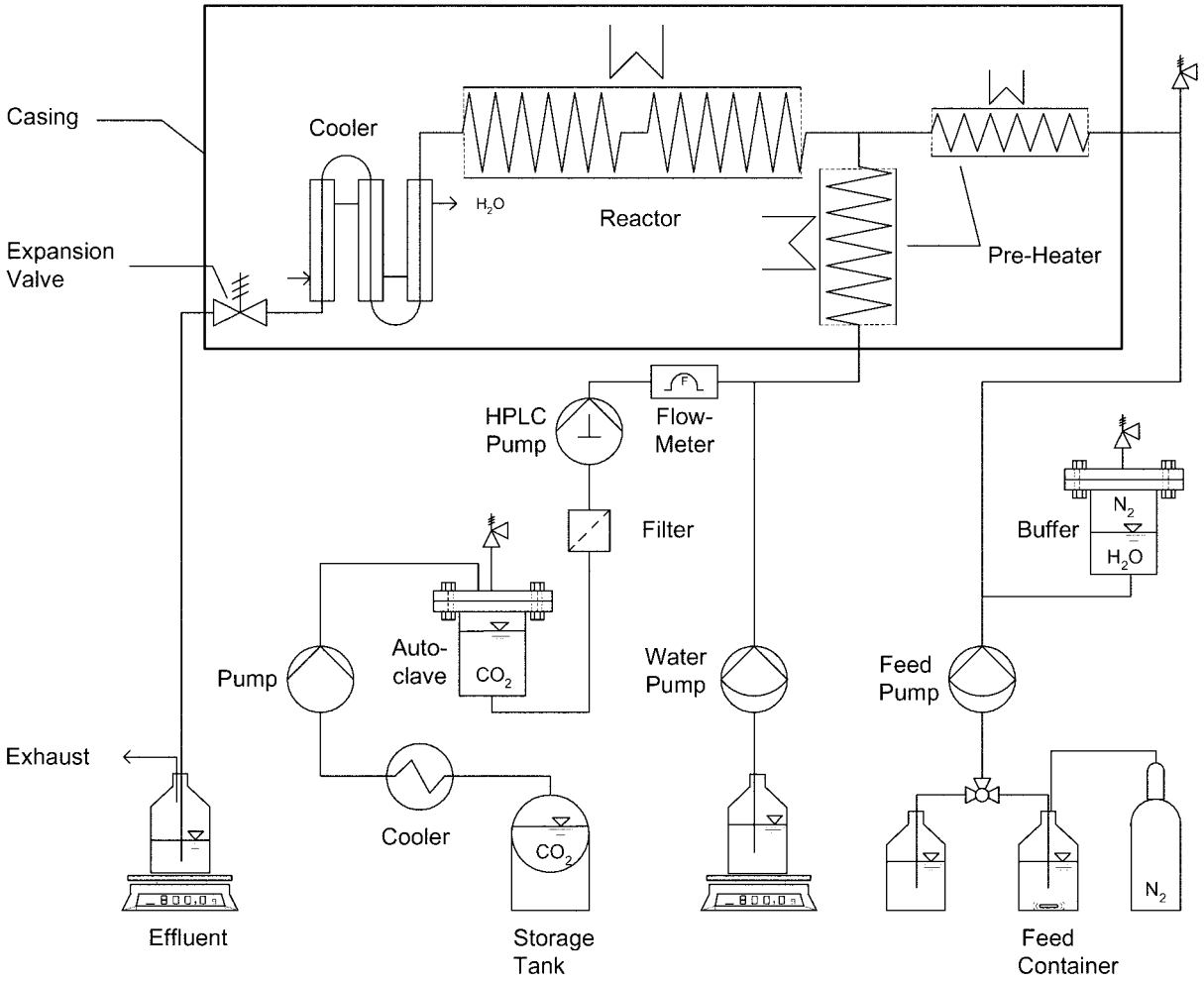

Figure 2. Experimental hydrothermolysis apparatus for subcritical liquefaction.

tem by means of a high-pressure membrane pump (LEWA EK1/V metering pump). Carbon dioxide is delivered from a storage tank, liquefied in a cooler and processed to an autoclave by means of an air-driven pump. Passing a filter unit, the carbon dioxide is introduced into the system by means of a HPLC pump. The amount of carbon dioxide is measured by a mass flow meter.

Fibrobacter succinogenes cultivation and fermentation

Fibrobacter succinogenes S85 (ATCC 19169) was originally isolated from the bovine rumen (Bryant & Doetsch 1954). A pure culture of this strain was grown anaerobically under 100% CO2 in a sterile basal medium containing (l^{-1}): 450 mg KH_2PO_4, 450 mg K_2HPO_4, 900 mg NaCl, 1.8 g $(NH_4)_2SO_4$, 90 mg $MgSO_4$, 90 mg $CaCl_2$, 3 mg $MnSO_4.6H_2O$, 0.3 mg $CoCl_2.6H_2O$, 8 mg $FeSO_4.7H_2O$, 0.25 mg biotin, 0.005 mg para-aminobenzoic acid, 500 mg cystein, 4 g Na_2CO_3 and a volatile fatty acid mixture (Gaudet et al. 1992). Of this pure culture, 400 ml was used to inoculate a stirred (100 rpm) fermentor (4 l) after a redox potential reduction at −350 mV and temperature equilibration at 39 °C. The pH, temperature and redox potential were measured on-line. Na_2CO_3 was added (4 g l^{-1}) by each substrate addition to stabilize the pH to a value of 6.9. Sterilised residual solids from the CSTR-reactor were added in batches (44 g in 2.8 l basal medium) at 1.5% DM to the basal *Fibrobacter* medium. Fermentations were performed for a period of 16 d.

Fermentation of hydrothermolysis and Fibrobacter *hydrolysates*

Following process sequence 1 (Figure 1), batch digestion tests were performed with both the hydrothermolysis and *Fibrobacter* hydrolysates in erlenmeyer flasks containing either 400 ml (small batch tests) or 800 ml (large batch tests) mixed liquour of the CSTR-reactor (depending on the organic strength of the substrate applied). The reaction times in the batch tests varied from 15 to 75 days. In all batch fermentation tests, a control was included which only contained mixed liquour from the CSTR main digester. The *Fibrobacter* hydrolysate and hydrothermolysis hydrolysate were added at various COD-concentrations (g COD l^{-1}) and fermented at various reaction times (d). The residues were added only once at the start of the experiment in amounts ranging from 40–150 ml, representing a COD-level of 0.1–2.7 g per test bottle. The biogas production and pH were continuously measured for each bottle.

Following process sequence 2 (Figure 1), a mesophilic 1.5 l fixed-bed biofilm reactor was employed to determine the biogas yield of the hydrothermolysis hydrolysate. The reactor was filled with 1 dm^3 of polypropylene carriers, with a specific surface of ca. 500 m^2 m^{-3}. To initiate the biofilm formation, 1 l of tapwater and 500 ml of sludge from the CSTR was added. Subsequently, the liquid was continuously recycled at an upflow velocity of 2 m h-1 and at daily basis 5 g COD l^{-1}.day^{-1} was dosed during a period of 10 weeks. After establishment of the biofilm, the hydrolysates were added to the fixed-bed biofilm reactor and continuously recirculated with an upflow velocity of 2 m h^{-1}. The biogas production and parameters as COD_t, COD_s, VFA and pH were followed on daily basis, during a total period of 21 days per experiment.

Analytical procedures and calculations

Van Soest analysis was performed for the quantitative determination of cellulose, hemicellulose and lignin fraction (Van Soest 1963; Van Soest et al. 1991). The COD, Kj-N, TAN, TSS (total suspended solids), VSS and ash-content of the digester influents and effluents were determined according to Standard methods (Greenberg et al. 1992). Biogas yields (%) were calculated on the basis of COD and VSS mass balances.

The volumetric biogas production was monitored by means of an electronic gas counter (Bergedorf, Hamburg-Harburg, Germany) with a resolution of 1 ml and by means of (acidified) calibrated water displacement columns for the CSTR digester and the batch tests, respectively. Biogas was analyzed for methane and carbon dioxide composition using a Varian 3800 gas chromatograph (PoraPLOT Q column (25 m, I.D. 0.53 mm, 20 μm), helium flow of 7 ml min^{-1}, isothermal 40 °C) equipped with a universal dual channel TCD-detector. Individual VFA concentrations (acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, isocaproic) were measured with a GC-FID AS800 gas chromatograph equipped with a FID-detector and N_2 as carrier gas. The column used was an Alltech (Deerfield, USA) EC-1000 (30 m, I.D.: 0.32 mm, d_f: 0.25 μm). Formic acid was measured by means of ion-exchange chromatography (Macherey und Nagel EC 200/4 Nucleosil 100 5NH2 column) equipped with a RI-detector. The analyses were conducted isothermally at 40 °C with an eluent mixture of 78% acetonitrile and 22% water at a flow rate of 0.7 ml min^{-1}.

The IC (inorganic carbon) buffer of the digester substrate and effluent was measured by means of titration with a Titrino 716 titrator (Metrohm, Switzerland). The sample was titrated as such with 0.1 N HCl (down titration profile) from the actual pH to pH 2.5 and data were automatically acquired and analysed (Van Vooren et al. 1999; Van de Steene et al. 2002).

SO_4^{2-}-S and PO_4^{3-}-P concentrations were measured before and after fermentation with a Metrohm IC 761 ion-chromatograph (IC) (metrosep A supp 5 (150 × 4 mm) column) with conductivity detector. Both influent -and effluent samples were diluted 100-fold after centrifugation and filtered over a 0.45 μm filter prior to injection.

Elementary composition analysis was performed with a CNS-analyser at 1100 °C (Leco-CNS-2000-Analyser). The dissolved carbon was determined by means of a TOC-analyser (Elementar "HighTOC + TN_b"). The liquefaction degree of the solids was then calculated by dividing the dissolved carbon after liquefaction by the total influent carbon.

HPLC-analysis with a ligand exchange chromatography (LEC) column (L = 300 m, i.d. = 7.8 mm) (Macherey Nagel, Nucleogel Sugar; RI detector) with an isothermal oven temperature of 72 °C (distilled water as eluent at 0.5 ml min^{-1}) was performed to determine the composition of the hydrothermolysis hydrolysate with respect to sugars and degradation products thereof.

Results

CSTR-digester performance and biogas yield

Table 2 summarizes the digestion parameters for the bioconversion of the raw substrate. The digester pH remained constant and total VFA concentrations during operation were low, indicating a stable digester performance for the given loading rate (1.5–2.5 g COD l^{-1} day^{-1}). Cellulose removal corresponded well with VSS and COD removal yields and amounted to 72% on average. The digester efficiently converted the majority (78% on VSS basis) of the organic substrate into biogas with average methane content of 65%. The specific gas production at a RT of 20 d was found to be relatively high (0.37 l biogas g^{-1} VSS added). In terms of nitrogen mass balances, an increase of the TAN-level in the effluent was observed while the Kj-N present in the influent (1.4 g l^{-1}) and the effluent (1.2 g l^{-1}) was nearly the same (Table 2). Hence, the majority of the organically bound nitrogen (mainly proteins) present in the raw substrate could be converted into ammonium species at a retention time of 20 days (Table 2).

Simulation and identification of the IC buffer titration results showed the presence of an IC buffer peak at a pH of 6.3 for both the digester influent and effluent. The bicarbonate concentration increased from 39 mM to 175 mM during biological treatment, whereas the ammonia nitrogen concentration increased from 0.4 to 1 g l^{-1}. For the influent, a third peak was observed but could not be identified (around pH-value 3.3), probably referring to the presence of high-weight proteins and/or acids. Overall, the PO_4^{3-}-P (mg l^{-1}) concentration of the raw substrate was high (500 mg l^{-1}) relative to domestic sewage. Influent SO_4^{2-}-S concentrations were moderate and resulted in 0.7% H_2S of the volume biogas produced.

Van Soest analysis showed that fibers accumulated in the mixed liquour of the digester, leaving a solid digester residue consisting of about 49% of fibrous matter (cellulose, hemicellulose and lignin). This fibrous solid residue, which accounted for 15–20% of the raw substrate on VSS and COD basis, was the subject of further liquefaction and subsequent biomethanisation (see Figure 1).

Batch fermentation tests with raw substrate showed that for an applied initial concentration of 1.85 g l^{-1} COD, biogas yields on COD and DM analysis varied from 25% for 10 d to 90% for 65 d reaction time. The increase in biogas yield as a result of an increase of the reaction time was most pronounced for the lower reaction times (10–20 days) (results not shown). The lower bioconversion found in the batch tests (70%) was not significantly different from the conversion yield found during continuous operation of the CSTR (78%) for the same reaction time (23 d).

Table 2. Performance data for the CSTR during operation at an average volumetric loading rate of 1.5–2.5 g COD l^{-1} day^{-1} and a RT = 20 d

Parameter	CSTR		
Retention time (d)	20		
Methane (%)	65% ± 3		
Specific gas production (l biogas g^{-1} VSS added)	0.37 ± 0.02		
Volatile fatty acids: (mg l^{-1} as HAc)			
Acetate	30 ± 5		
Propionate	3 ± 1		
Butyrate	1 ± 1		
Isobutyrate	1 ± 1		
Valerate	0		
Isovalerate	10 ± 2		
Capronate	0		
Isocapronate	1 ± 1		
Total VFA (digester)	46 ± 7		
pH	7.4 ± 0.2		
Mass balance parameters	**Influent (g l^{-1})**	**Effluent (g l^{-1})**	**Removal (%)**
TSS	28	7.5	73 ± 2
VSS	24	4.8	78 ± 2
COD	21	5.9	80 ± 8
Cellulose	5.9	1.6	72 ± 10
Total Kjeldahl Nitrogen	1.4	1.2	14 ± 4
Total Ammonia Nitrogen	0.4	1	−150 ± 2

Figure 3 shows the CSTR biogas yield at different retention times. At a lower RT (15 d), only 35–50% of the COD of the raw substrate could be transformed into methane. For a more conventional RT of 20–25 d, on average 70–78% of the raw feed could be converted into biogas. At a RT as high as 60–75 d, the raw substrate was converted into biogas with a yield of 80–85%.

Fiber liquefaction by hydrothermolysis

Despite the variable temperatures and pressures applied, the carbon liquefaction efficiencies for one and

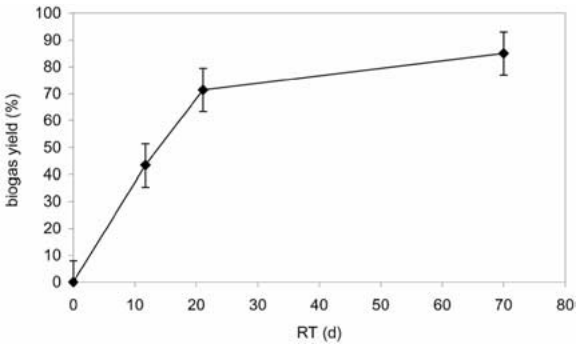

Figure 3. CSTR biogas yield from raw substrate at 3 different retention times. 100% biogas yield corresponds to a conversion of 0.5 l biogas for 1 g COD. Standard deviations are based on the biogas yields at 3 different initial concentrations (1.1, 1.8 and 2.8 g/l COD).

Table 3. Carbon liquefaction efficiencies of the CSTR effluent solids at various conditions (T = 301–406 °C, p = 233–264 bar). No 1–6: carbon liquefaction of various effluent solid batches without CO2 saturation; No 7: carbon liquefaction of effluent solids with 50% CO_2 saturation. All experiments were performed with the same batch of CSTR solids except experiment No 1

No	T [°C]	P [bar]	RT [s]	Carbon liquefaction [%]*
1	360	240	25.1	73.9
2	366	238	39.7	56.4
3	360	233	38.8	57.1
4	301	250	87.2	44.8
5	319	247	45.2	58.7
6	406	264	>35	57.2
7	341	238	50	83.4

* Calculated as $C_{\text{soluble out}}/C_{\text{in}} * 100$.

the same batch of CSTR solids varied between a relatively narrow range of 44–57% (Table 3, run No 2-6). Experiment No 2 and No 3 show that the reproducibility of the thermal reactor was high (less than 2% difference). The fourth experiment was performed at a lower temperature of 300 °C and yielded a significantly lower degree of liquefaction of 45%, though the residence time of 87 s was much longer than for the other runs. Even an increase in temperature to 406 °C (experiment No 6), which is well above the critical temperature of pure water, did not result in a higher degree of liquefaction within the residence time employed in the experiments. This implies that for this particular batch, about 40% of the carbon was very difficult to liquefy without a further increase in residence time. However, a significantly higher carbon liquefaction yield was reached for experiment No 1. This demonstrates that the solids batch used for No 1 presumably contained less inert material compared to the solids batch used for No 2–7.

In order to increase the degree of conversion, carbon dioxide was added to the system (No 7) by which the pH of the influent was considerably lowered. Carbon liquefaction yields higher than 80% could be reached with the same solids batch as No 2–6 by equilibrating the liquor with 50% CO_2 (Table 3).

HPLC analysis of the hydrolysates at a hydrothermolysis temperature of 310 °C and 350 °C showed that saccharides were present only in very small concentrations ($<50\ \text{mg}\ \text{l}^{-1}$). Raffinose, maltose, fructose, glucose, pyranose, and hydroxyl-methylfurfural, could be detected at 310 °C. For these conditions, distinct peaks were found at residence times shorter than that of raffinose, which are due to the formation of oligo-saccharides. For the 350 °C hydrolysate, oligo- and mono-saccharides could not be detected. Instead, pyranose and hydroxymethylfurfural were produced in significantly higher amounts. Beside sugars, formic and acetic acid accounted for up to 20% of the total soluble carbon. The concentrations of higher acids were negligible. Due to the production of acidic degradation products during hydrothermolysis, the pH of all thermally treated influents decreased to values of 4.1–4.4. Essentially all nitrogen (95–100%) initially present in the solid phase (measured by elementary analysis) could be converted to water-soluble components in the course of the hydrothermal degradation. NH_4^+-N and NO_3^--N amounted to about 60% of the total nitrogen detected in the liquid phase after hydrothermolysis. The remaining nitrogen fraction (40%) could not be identified but it is assumed that this fraction is present in the form of other oxidised nitrogen species. The contribution of free amino acids to this unidentified fraction can be considered to be very minor, since it was shown that amino acids are instable and subject to consecutive reactions at the temperatures applied in the experiments (Walter et al. 1967). This finding was supported by own studies on the decomposition behaviour of bovine serum albumin in near-critical water.

With regard to the gas phase, no other components than nitrogen (added at the start) and carbon dioxide could be detected in any of the experimental runs. As can be inferred from the results of these measurements, the amount of carbon in the gas phase only had a minor contribution (2–3%) to the total carbon introduced into the system. In accordance with the high temperatures and pressures applied, the effluents of the

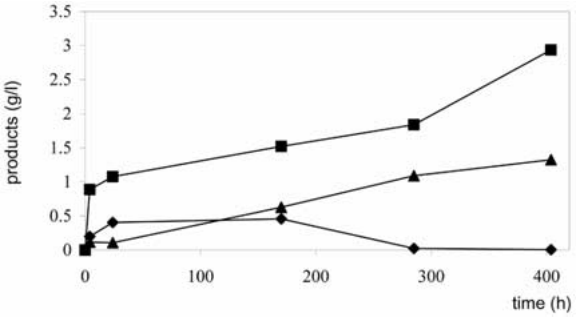

Figure 4. Production of organic acids during *Fibrobacter succinogenes* fermentation on CSTR- effluent solids at 15 g l^{-1}. Key: ■ = acetate, ▲ = propionate, ♦ = succinate.

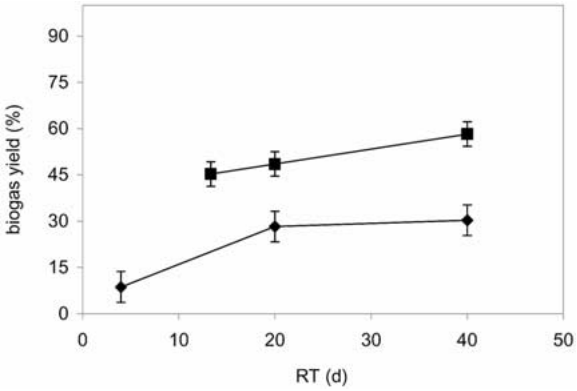

Figure 5. Biogas yields of the undiluted hydrothermolysis hydrolysate (■) and the *Fibrobacter* hydrolysate (♦) in batch fermentation tests at various reaction times (triplicate tests). Initial concentrations applied were 0.2–0.75 g l^{-1} COD for the hydrothermolysis hydrolysate and 2–3 g l^{-1} COD for the *Fibrobacter* hydrolysate.

hydrothermal treatment were found to be completely sterile.

Fiber fermentation by Fibrobacter succinogenes

Fed-batch fermentations were performed by the rumen bacterium *Fibrobacter succinogenes* on the CSTR-effluent solids. The overall liquefaction yield for the recalcitrant digester solids was found to be 41% on DM-basis (data not shown).

Figure 4 shows the typical profile of degradation products formed during *Fibrobacter* fermentation of the CSTR-effluent solids with substrate addition at 0 h and 180 h. During the first hours of culture, mainly succinate and acetate were produced as sole metabolites causing a concomitant small decrease in pH (0.3–0.5 units). After 24 hours, the production of succinate stopped and other VFA began to be produced (mainly acetate and propionate). Final VFA concentrations were highest for acetate and propionate corresponding to values up to 3 g l^{-1} and 1.1 g l^{-1} respectively.

Gas analysis showed that CO_2 was the only gaseous compound produced during *Fibrobacter* fermentation in quantities representing less than 10% of the input carbon.

Biogas yields of hydrothermolysis and Fibrobacter hydrolysates

CSTR conversion efficiencies for the hydrothermolysis hydrolysate were significantly higher compared to the *Fibrobacter* effluent (Figure 5). For the hydrothermolysis hydrolysate, biogas yields of 48% and 60% were observed at a reaction time of 20 days and 40 days respectively. As can be derived from Figure 5, about 30% of the COD of the *Fibrobacter* residue could be converted into biogas at a reaction time of 20 days or more. Hence, the transformation of the hydrothermolysis hydrolysate was dependent on the retention time and proceeded at a higher efficiency compared to the *Fibrobacter* hydrolysate (Figure 5).

Figure 6 shows the COD removal and biogas production of the hydrothermolysis hydrolysate following process sequence 2 (Figure 1) employing an upflow biofilm reactor. During the first 7 days, 59.5% of the influent COD could be converted into biogas (Figure 6A) with an average methane content of 65%. The VFA-content of the hydrolysate was low (VFA_{total} = 87 mg l^{-1}) and was completely removed after 1 day of fermentation. The cumulative biogas production mounted to 0.75 l after 7 days or an average biogas production rate of nearly 0.5 l per g COD removed (Figure 6B).

Based on the biogas yields from the raw substrate and the liquefied CSTR solids by hydrothermolysis and *Fibrobacter* fermentation, the overall conversion efficiencies for the raw substrate were calculated (Figure 7). By applying the sequence CSTR digestion/*Fibrobacter succinogenes* digestion/CSTR digestion, an overall biogas yield of 82% could be reached. The use of hydrothermolysis in combination with CSTR digestion (column C and D) increased the biogas production with 10%, giving rise to an overall biogas yield of 90%. The overall biogas yield found in column D (92%) following process sequence 2 (Figure 1) was however not significantly higher compared to column C (Figure 7).

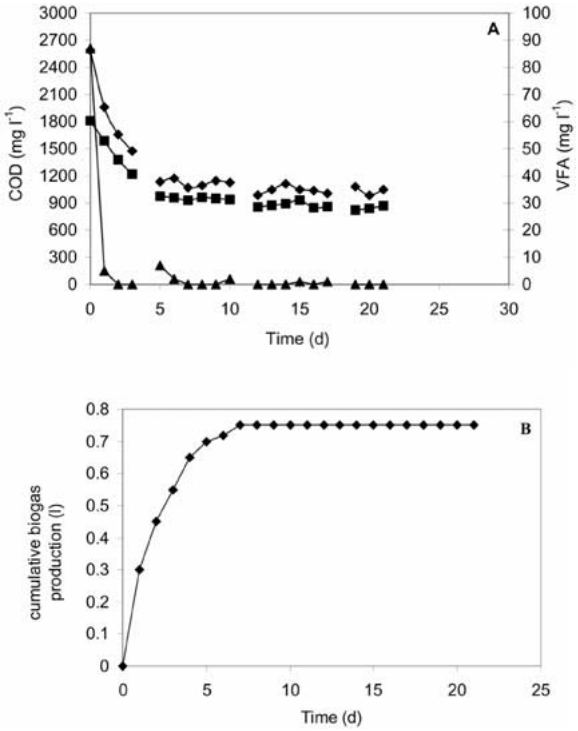

Figure 6. Anaerobic mesophilic digestion of diluted hydrothermolysis hydrolysate with an upflow methanogenic biofilm reactor. The hydrolysate was recycled through the biofilm reactor for a period of 21 days at an upflow velocity of 2 m h^{-1}. Figure A: ***left Y-axis***; ♦ = COD_{total}, ■ = $COD_{soluble}$, ***right Y-axis***; ▲ = Total VFA. Figure B: corresponding cumulative biogas production.

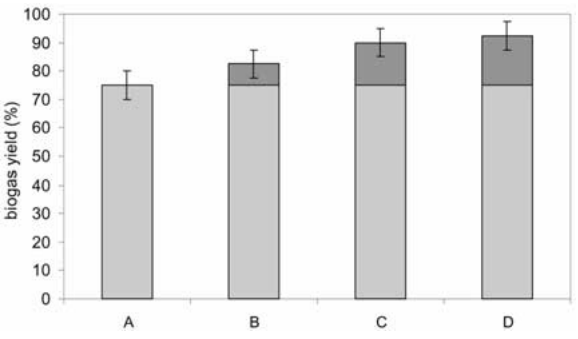

Figure 7. Biogas yield of raw substrate for mesophilic digestion (A), mesophilic digestion combined with *Fibrobacter succinogenes* fermentation (B), mesophilic digestion combined with hydrothermolysis (C) and mesophilic digestion combined with *Fibrobacter* fermentation and hydrothermolysis (D). Key: ***Light grey*** = biogas yield of CSTR; ***Dark grey*** = biogas yield after *Fibrobacter* fermentation and/or hydrothermolysis. The material not converted to biogas was inert to biological transformation.

Discussion and conclusions

In this work, the nearly-complete anaerobic conversion of a life support substrate consisting of food waste, green algae and faeces was studied. It was shown that a VSS decrease of 78% and a specific gas production of on average 0.37 l biogas per g VSS added could be reached by means of a one-stage CSTR-type mesophilic digester (RT = 20 d). These performance data are in good accordance with reported conversion efficiencies for non pretreated biosolids (e.g., manure) and lignocellulosic waste (e.g., rice straw) with biogas yields around 50% on COD basis (or 0.2–0.25 l CH_4 g^{-1} VSS) (Bonmati et al. 2001) and specific biogas productions in the range of 0.39 L biogas g^{-1} VSS (Zhang & Zhang 1999) respectively.

The biogas yield of the CSTR during continuous operation (78%) was statistically in the same range compared to the biogas yields calculated from the batch fermentations (70%). The slightly lower value for the batch test was most probably caused by the subtracted value from the control (mixed liquor without COD loading) used in the batch tests.

The organic waste employed had a high nutrient content with a C/N ratio in the range of 10, mainly due to the presence of faecal matter (20% DM) and the green alga *Spirulina platensis* (10% DM). Hansen et al. (1998) reported that anaerobic digestion of pig manure was inhibited at a pH of 8 corresponding to a free ammonia concentration of 1.1 g l^{-1} or more which caused a decrease in methane yield. Despite the relatively high TAN concentrations in the effluent (1 g l^{-1}), methanogenesis was never inhibited at the applied OLR, presumably because of the lower digester pH (7.4 on average). The IC buffer constituted together with the total ammonia (NH_3 and NH_4^+) a high buffer capacity in the digester, which explained the pH stability of the digester. Since the average pH of the reactor was 7.4, part of the total ammonia (TAN) was present as ammonia and could possibly be transferred to the gas phase. However, it is generally accepted that up to a pH of 7 the total ammonia is only present under the form of NH_4^+ (Perrin, 1965). Moreover, no significant losses in nitrogen could be detected by TAN- and Kj-N analysis. Therefore, it is assumed that nitrogen losses by means of the produced biogas were minor.

A significant amount of ortho-phosphate was taken up in the digester since the effluent ortho-phosphate concentration was only 1/3 of the influent concentration. Phosphorous is generally much less mobile

than nitrogen and can be strongly adsorbed to organic matter and/or sludge (Nowack & Saladin 2000). The enrichment of ortho-phosphate in the digester might thus be explained by adsorption on particulate matter.

The pronounced influence of the RT on the biogas yield (Figure 3) gives evidence that after the first 20 d of digestion, the biogas production significantly reduces due to the exhaustion of readily available substrate (day 25–40). Since the digester substrate contained a considerable amount of lignocellulose, it can therefore be hypothesized that the hydrolysis of particulate organic matter (e.g., lignocellulose) became rate-limiting during the course of digestion. Hydrothermolysis of the CSTR solids resulted in high carbon liquefaction yields, varying between 44–83% (Table 3). Sakaki et al. (1996) also showed that cellulose decomposes very rapidly in catalyst free hot compressed water of around 300–400 °C, and that the resulting water-soluble products quickly decomposed. The data suggest that adding carbon dioxide to the influent might stimulate hydrolysis kinetics, as can be inferred from the increased degree of liquefaction (Table 3). Due to the increased solubility of carbon dioxide in water at elevated temperatures and pressures, the addition of carbon dioxide can serve as a means to lower the pH-value without the need of mineral acids. By decreasing the pH, many acid catalysed reactions like the hydrolysis of glycosidic bonds can be greatly accelerated (Lehninger 1975). This approach bears the advantage of easily recovering the carbon dioxide in the gas phase, such that additional downstream unit operations like neutralisation and precipitation steps become superfluous (Liu 2000).

The hydrolysis temperature during hydrothermolysis played a major role in the formation of degradation products. The production of hydroxymethyl furfural, which is known to be a potential inhibitor of methanogenesis (Rivard & Grohmann 1991), was promoted at higher temperatures. Other potential fermentation inhibitors could however not be detected. Subsequent digestion efficiencies for the undiluted hydrothermolysis hydrolysate were significantly higher at higher RT (Figure 5). Different from CSTR fermentation, the digestion of the diluted hydrolysate derived from the most recalcitrant solids occurred without any lag phase in the upflow biofilm reactor with a COD removal of 59% (Figure 6A) and a high biogas yield (Figure 6B). These results indicate that the toxicity of the hydrothermolysis hydrolysates to the bacterial consortia in both methane reactors was of no concern for the reliability of the system within the tested time limits. Addition of carbon dioxide to lower the pH of the influent suspension seems to have a catalyzing effect on the hydrolytic degradation and will therefore be systematically investigated in further studies by varying the operating condition, including different degrees of carbon dioxide saturation. The rumen bacterium *Fibrobacter succinogenes* followed by subsequent methanogenesis was able to convert 30% of the CSTR solids into biogas (Figure 5). Initially, *Fibrobacter succinogenes* mainly produced acetate and succinate as dominant fermentation products (Figure 4). In other studies, where *Fibrobacter* was grown in cellobiose-limited conditions (5 mM), also succinate and acetate were produced (Maglione et al. 1997; Fields et al. 2000). However, the reconsumption of succinate and the production of large amounts of acetate (up to 3 gl^{-1}) and propionate (1.1 gl^{-1}) are rather unusual. The production by *Fibrobacter succinogenes* of large amounts of acetate from succinate has already been demonstated by in vivo ^{13}C NMR studies (Bibollet et al. 2000). This phenomenon of reversion of the succinate pathway was observed as well in adherent and non-adherent cells and was favoured by high nitrogen concentration.

The presented thermal/biochemical conversion system demonstrates that a life support organic waste can nearly be completely converted (90% biogas yield) into energy-rich methane gas, leaving a mineral- and nutrient rich effluent and carbon dioxide suitable for the growth of secondary foods in space.

Due to its efficiency, the presented system is highly attractive for life-support systems where hygienic, rapid and total conversion of organic waste is of major importance (Fulget et al. 1999). The recovery of energy from the the high temperature hydrothermolyis step is in principle feasible, the heat integration of the complete system being an issue which has to be solved in future work.

Acknowledgements

The authors acknowledge the ESA (European Space Agency) for the funding of the project “A total converting and biosafe liquefaction compartment for MELiSSA” (Micro-Ecological Life Support System Alternative).

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