Spruce bark beetles as a biological waste that can be processed in a biogas plant

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Abstract: Biogas plays an important role in reducing the amount of biowaste that is converted to methane which is usable by multiple pathways in the energy sector. Bark beetles are among the most important pests of spruce forests in Europe. Principal measures of managing bark beetle populations include trapping them with pheromone traps. Biomass of bark beetles collected by the traps constitutes a waste of forest resources that could potentially be processed in biogas plants. The aim of this study was to provide information about the trapping of bark beetles by pheromone traps in Tatra National Park during 2005–2018 and to verify the energy potential of a combination of liquid digestate and bark beetle biomass collected by pheromone traps. The bark beetle biomass is a seasonal small-volume nitrogen-rich material which could be processed at agricultural biogas plants. The biochemical methane potential batch test performed at 40 °C showed a high degree of digestion of milled beetles within 40 days, with a methane production of 0.242 Nm³ per kilogram of total solids (TS). The single-stage low-solids mesophilic fed-batch anaerobic digestion of untreated beetles was carried out in a lab-scale rotary drum reactor. Within 52 days, the average organic loading rate was 1.019 kg VS m⁻³ day⁻¹, where VS stands for volatile solids. The specific biogas and methane production reached 0.218 Nm³ kg⁻¹ TS and 0.140 Nm³ kg⁻¹ TS, respectively. The cuticles of the beetle bodies were not obviously disturbed by the fermentation process. The digestate was quite rich in nitrogen, but the other element contents did not differ too much from fertilizer made from manure or phytomass.

Key words: Biochemical methane potential, fertilizer, *Ips typographus*, low solids anaerobic digestion, pheromone trap, *Pityogenes chalcographus*

1. Introduction

Bark beetles are a natural component of forest stands. They play an important role in the natural forest cycle by decomposing wood, thus sustaining nutrient availability of the forest ecosystem in the long term (Christiansen et al., 1987; Zahradník and Knížek, 2007). On the other hand, bark beetles are among the most significant pests of spruce forests in Europe. Spruce forests are linked to about 30 species of insects, of which 3–4 are serious pests (*Ips typographus* L., *I. amitinus* Eichhoff., *I. duplicatus* Sahlberg, and *Pityogenes chalcographus* L.). In Slovakia, the most dangerous bark beetles of spruce forests are *I. typographus* and *P. chalcographus*. They are well-defined and distinguishable species, recognized mainly as pests of spruce (*Picea* spp.) in Eurasia. These insects normally establish on fallen trees but can also mass-attack healthy trees, killing millions of spruces (Jeger et al., 2017). The last decade of the 20th century was marked by several disastrous windstorms in Central Europe, which gave rise to an enormous propagation of bark beetles in the affected

spruce forests. In managed forests, several strategies for bark beetle control have been applied to reduce its population size and to prevent the emergence of outbreaks (Nikolov et al., 2014; Havašová et al., 2017). The principal aim of managing bark beetles is to minimize attacks on living trees. Measures most commonly applied for this purpose include the trapping of bark beetle adults by the installation of pheromone traps or trap trees.

In the Slovak Republic, the extent of bark beetle damage is huge; in the years 1960–2014, as many as 26.9 million m³ of wood were destroyed. Over 1.2 million m³ of wood infested with spruce bark beetle were processed in 2015, and 2.7 million m³ in 2016. Overall, 76,348 pheromone traps were installed (65,593 traps for *I. typographus* and 10,755 for *P. chalcographus*) in Slovak spruce forests in 2016 (Vakula et al., 2017). The flight activity of *I. typographus* depends on the temperature—minimum air temperature for flight is 16.5 °C and optimum temperature is between 22 °C and 26 °C (Wermelinger, 2004)—and occurs over a period of about 4 months (mainly between 15 April and

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15 August). The efficiency of pheromone traps is usually estimated from 2% to 30% (Facoli and Stergulc, 2004).

The average fresh weight of a single *I. typographus* (if we consider the ratio of females/males to be equal) is 7.35×10^{-3} g (Bednarz and Kacprzyk, 2012) and that of a *P. chalcographus* individual is 3.06×10^{-4} g (Kacprzyk and Bednarz, 2015). The total weight of a single beetle is negligible, but with large quantities, this waste biomass could somehow be useful. The mass of dead spruce bark beetles collected from the pheromone traps constitute a waste of forest resources.

Biogas is one of the most widely used types of renewable energy in European Union countries. Biogas is expected to play an important role in reaching the future energy policy targets of the European Union (EU). However, the sustainability of biogas substrates has been recently discussed critically due to the increasing shares of agricultural land used for energy crop production. In the EU, political targets for climate and energy in 2030 are 40% reductions in greenhouse gas emissions, 27% renewable energy installed capacity for the entire EU energy supply, and 27% improvement in the energy efficiency (Meyer et al., 2017).

The aims of this paper are (1) to verify the wet anaerobic production of biogas, containing methane and liquid, fertilized from bark beetles collected from pheromone traps; (2) to provide information on potentially processing this seasonal biomass in a biogas plant of the agricultural type; and (3) to summarize catches of bark beetles in forests of Tatra National Park in Slovakia during the period of 2005–2018.

2. Materials and methods

2.1. Substrate and pheromone trapping data

The spruce bark beetle quantity was monitored with pheromone traps from 2005 to 2018, in Tatra National Park (TANAP). The monitoring pheromone traps were available in the High Tatras (Table 1). Traps were baited with commercial lures for attracting the spruce bark beetle: IT ECOLURE (Fytofarm s.r.o., Karlova Ves, Slovakia) (with an active substance of S-cis verbenol) for *I. typographus* and PC ECOLURE (Fytofarm s.r.o., Karlova Ves, Slovakia) (with chalcogran as an active substance) for *P. chalcographus*. The traps were checked at 7–10 day intervals and the number of trapped beetles was measured by a volumetric method using the following coefficients: 1 mL of beetle biomass equaled 40 individuals of *I. typographus* and 600 beetles of *P. chalcographus* (Galko et al., 2016).

The biomass of 15 kg of dead bark beetles was used for experiments. The substrate contained a mixture of both mentioned species plus a small proportion of digested bast and particles of wood and bark.

2.2. Inoculum

Liquid digestate (reacting suspension mixture) from the first-stage anaerobic fermenter of the agricultural biogas station Pustějov II (Zemspol Studénka Ltd.; Moravian-Silesian Region, Czech Republic), which mainly treats cattle slurry and corn silage, was used as the inoculum source. The temperature of the suspension having approximately 8 wt% of total solids decreased from 40 °C to approximately 30 °C during the transfer. The suspension was filtered through a sieve with 0.8×0.8 mm apertures. The filtrate having 5.57 wt% total solids, C:N ratio of 8.6, VFA/TIC ratio of 0.25, and pH-value of 8.0 was used as inoculum; detailed physical, chemical, and microbiological analyses of inoculum, substrate, and digestate were analyzed (Tables 1 and 2).

2.3. Substrate pretreatment

For the biochemical methane potential (BMP) test, the substrate was not dried, and was milled using an IKA Tube Mill Control analytic mill (IKA Works, Inc., Wilmington, NC, USA). Typical particle size was around 0.05 mm. For the fed-batch digestion test, the substrate was used without pretreatment.

2.4. Biochemical methane potential batch test

A discontinuous test of biochemical methane potential was performed according to the EN ISO 11734:1998 standard (EN ISO 11734) and VDI 4630:2016 (VDI 4630) on the apparatus consisting of a 1-L reaction bottle, 1.2-L gas burette, and 2-L expansion bottle for a closing saturated salt solution (Figure 1). Reaction bottles were placed in a thermostatic water bath at 40 ± 0.2 °C. Gas burettes were placed in the laboratory fume hood at 20 ± 2 °C. To determine the endogenous production of biogas and methane (specific production related to the inoculum), 2 reactors were used, and for the addition of substrate, 2 more reactors were added. In the period of 40 days, the ambient temperature (temperature of biogas), barometric pressure, and increase of biogas volume were measured once each working day at 8:00. Gas volume was calculated for standard conditions (0 °C, 101.325 kPa). When a sufficient quantity of biogas was collected in the burette (>150 mL), analysis of biogas composition was performed using a portable analyzer. The methane content was corrected to residual biogas aeration. The weekend data on biogas volume and methane content were interpolated linearly. The H₂ content was measured only to demonstrate the relatively low inoculum load, and the H₂S content was measured to detect possible inhibition of biogas production.

2.5. Fed-batch digestion test

Due to the very low density of the beetles, it was obvious that they would form a layer at the batch level and that it would be impossible to continuously collect representative samples of digestate from the bioreactor. For this reason, the main digestion test was designed as a fed-batch process.

Table 1. Physical and chemical parameters of inoculum, spruce bark beetle biomass, and anaerobic digestate.

Parameter	Mark	Unit	Inoculum	Spruce bark beetles	Anaerobic digestate
Density (actual TS)	ρ	kg m^{-3}	1033	1239	1046
Material density (100% TS)	ρ_{TS}		1588	1483	1560
Bulk density (mean TS)	ρ_{B}		-	200–250	-
Hydrogen exponent	$\text{pH-H}_2\text{O}$	-	8.0	8.2–9.0	8.6
Volatile fatty acids $\text{C}_2\text{--C}_5$	VFA	wt. %	0.33	-	0.85
Total inorganic carbonate	TIC		1.35	-	2.36
Total solids	TS		5.57	35–92 (mean 49.40)	8.15
Volatile solids	VS_{TS}	% TS	67.80	84–90 (mean 87.80)	71.64
Total combustibles	$\dot{\text{C}}$		68.27	87.41	73.65
Volatile combustibles	V		55.95	71.43	58.31
Fixed carbon	FC		12.32	15.99	15.34
Ash	A		31.73	12.59	26.35
Lipids	CL		2.0	-	8.9
Simple carbohydrates	CH		0.6	-	0.1
Starch	ST		1.5	-	0.1
Crude fiber	CF		8.6	-	7.7
Crude protein	CP		1.0	-	3.9
Nitrogen-free extract	NFE		25.1	-	0.9
Nitrogenous substances	NC		27.6	-	44.3
Lignin	L		2.9	-	4.6
Carbon	C		38.11	47.76	40.94
Hydrogen	H	$\text{mg kg}^{-1}\text{TS}$	4.54	6.35	5.01
Nitrogen	N		4.44	10.81	6.09
Oxygen	O		20.98	22.12	21.39
Sulfur	S		0.21	0.37	0.21
Ammonia nitrogen	N-NH_4^+		2.53	-	3.71
Nitrate nitrogen	N-NO_3^-		0.02	-	0.02
Phosphorus	P		1.00	-	1.51
Calcium	Ca		2.02	-	2.72
Potassium	K		3.41	-	1.20
Magnesium	Mg		0.56	-	0.63
Arsenic	As		<0.5	-	<0.5
Cadmium	Cd		1.6	-	2.0
Cobalt	Co		<2.5	-	<2.5
Chrome	Cr		4.4	-	10.5
Cuprum	Cu		106.0	-	85.0
Iron	Fe	$\text{MJ kg}^{-1}\text{TS}$	1487	-	1020
Nickel	Ni		10.3	-	11.4
Lead	Pb		4.1	-	<2.5
Zinc	Zn		410.0	-	126.0
Molybdenum	Mo		2.5	-	<0.5
Mercury	Hg		0.04	-	0.03
Selenium	Se		<0.4	-	<0.4
Gross calorific value (at constant volume)	$q_{\text{Vgr,TS}}$	$\text{MJ kg}^{-1}\text{TS}$	17.59	21.35	18.02
Theoretical biogas production	$B_{\text{t,V}}$	$\text{Nm}^3 \text{kg}^{-1}\text{TS}$	0.719	0.901	-
Theoretical CH_4 content in biogas	$\%_{\text{CH}_4\text{t}}$	vol.%	54	54	-
Theoretical CH_4 production	$M_{\text{t,V}}$	$\text{Nm}^3 \text{kg}^{-1}\text{TS}$	0.386	0.485	-

Table 2. Microbiological parameters of inoculum, spruce bark beetle biomass, and anaerobic digestate.

Parameter	Mark	Unit	Inoculum	Spruce bark beetles	Anaerobic digestate
Enterococci	ENT	CFU g ⁻¹ TS	2 · 10 ⁵	-	1 · 10 ⁴
Thermotolerant coliforms	TTC	CFU g ⁻¹ TS	5 · 10 ¹	-	5 · 10 ¹
Escherichia coli	EC	CFU g ⁻¹ TS	5 · 10 ¹	-	5 · 10 ¹
Salmonella	SLM	posit./negat.	negative	-	negative
Listeria monocytogenes (25 g) vzorku)	LM	posit./negat.	negative	-	negative
Yeasts	Y	CFU g ⁻¹	1 · 10 ¹	-	1 · 10 ¹
Fungi	F	CFU g ⁻¹	1 · 10 ¹	-	1 · 10 ¹
Thermophilic microorganisms	TM	CFU g ⁻¹	1 · 10 ⁶	-	1 · 10 ⁶
Mesophilic bacteria	MB	CFU g ⁻¹	1 · 10 ⁶	-	1 · 10 ⁶
Psychrophilic bacteria	PB	CFU g ⁻¹	4 · 10 ⁶	-	5 · 10 ⁶

Figure 1. Biochemical methane potential test apparatus.

The single-stage mesophilic (40 ± 1 °C) digestion was carried out in the rotary drum-type bioreactor INFORS HT Terrafor IS (INFORS HT, Basel, Switzerland) with a warm water jacket for 52 days. The drum was slightly modified for securing immersion of the very light substrate beneath the surface of the batch. The 4 original small paddles in the upper and lower domed bottoms were removed, and 1 stationary ribbed scraper blade was installed on the drum shaft head. A 6-kg stainless steel crushing roller was inserted (Figure 2).

The process was started by discontinuous digestion of liquid inoculum at 5.57 wt% of total solids. Manual dosing of the substrate was done once a day (excluding weekends) from the seventh day onward. Drum rotation speed was kept constant at 1.0 min^{-1} throughout the test. The drum axis was set in the horizontal plane. When handheld substrate dosing was carried out every working day, the drum was lifted by 45°. The digestate was analyzed at the end of the test.

Figure 2. Drum-type bioreactor with a ribbed scraper blade and crushing roller.

2.6. Measurements and analysis

The bulk density of the sample was checked directly in the storage vessel with a total volume of 0.03 m³, then measured in the 2-L measuring cylinder. The density was measured using a Thermo Scientific Pycnomatic ATC semiautomatic pycnometer (Thermo Scientific, Waltham, MA, USA) with helium gas. The potential of hydrogen (pH) (EN15933), total solids content determined by drying at 105 °C to constant weight (TS) (EN15934), and loss on ignition of the total solids at 550 °C to constant weight (VS_{TS}) (EN15935) were determined in the inoculum, substrate, and final digestate. The inoculum, substrate, and digestate were also analyzed for total combustibles, volatile combustibles, fixed carbon, and ash content according to the coal standard (ASTM). The pH was measured by WTW 340i with SenTix 41 probe. The TS parameter was determined using a KERN DLB160 3A moisture analyzer with halogen lamp (KERN, Balingen, Germany). Ignition was carried out on a LECO TGA 701 thermogravimetric analyzer (LECO, St. Joseph, MI, USA). The content of the C, H, N, and S was determined with a LECO Truspec CHN+S 628 analyzer (EN15104). The total carbon content was checked using a thermal analyzer LECO RC612 TOC with IR CO₂ detection (EN 15936). The gross and net calorific value was determined on a LECO AC 600 semiautomatic isoperibolic calorimeter (EN14918). The VFA/TIC (FOS/TAC) ratio of the anaerobic batch was measured according to the Lossie method (Lossie and Pütz, 2008) using a HACH Lange TIM BIOGAS V02.2 automatic titration station (Hach Co., Loveland, CO, USA).

The continuous volumetric measurement of raw biogas leaving through the pipe in the Terrafor IS reactor stationary shaft was performed by water-calibrated drum-type flow meter Ritter TG05/5 (transparent PVC, 0.001–0.060 m³ h⁻¹, water calibrated) (Ritter, Bochum, Germany). The composition of raw biogas was measured once a working day (prior to substrate dosing) by a portable infra-red/electrochemical analyzer Geotech Biogas 5000 (Geotech, Warwickshire, UK) (CH₄ 0%–70% ± 0.5%, CO₂ 0%–60% ± 0.5%, O₂ 0%–25% ± 1.0%, H₂ 0–2000 ppm ± 2.0% FS, H₂S 0–5000 ppm ± 2.0% FS). The list of standards is given in Table 3.

3. Results and discussion

The presented study gives information about catches of spruce bark beetles in the years 2005–2018 and the potential for using the trapped mass of beetles in anaerobic digestion. During the whole monitoring period, the average of the catches (Table 4) was 39.8 million pcs of *I. typographus* (292.7 kg) in Tatra National Park. In individual years, the population density of spruce bark beetle has gradually changed. The highest quantity was recorded in 2009 (111 million pcs/816 kg of IT) in TANAP,

and the lowest quantity in 2014 (2.1 million pcs/15.4 kg). The further rise of spruce bark beetle population density is assumed. Usually, the occurrence of spruce bark beetles is highest in the third and fourth year after a windstorm.

The pheromone traps could be a useful tool for monitoring *I. typographus* populations and for assessing the risk of tree attacks (Lindelöw and Schroeder, 2001). The population curve of spruce bark beetles fluctuates with climatic factors (Galko et al., 2016). There is a decreasing tendency to use commercially available pheromone traps, but the risk of bark beetle outbreaks in forests is still increasing due to ongoing climate change (Bentz and Jönson, 2015), particularly the lack of precipitation and increasing drought episodes (Kolb et al., 2016). The effectiveness of pheromone traps has been analyzed by many authors and varies significantly depending on the methods used for evaluation. Zahradník et al. (1995) recaptured 10.4%–1.9% of released beetles; Franklin et al. (1999), 2.3%–7.0%; Helland et al. (1984) recaptured 7.3%–10% in a radius of 500 m; however, Anderbrant et al. (1985) recaptured only 0.2% from a radius of 30 m. Higher effectiveness was reported by Weslien and Lindelöw (1990) (18%–37%), Zúmr (1992) (16%–27%), and Zúmr (1990, 1991) (37.6%–77%). The above data are markedly inconsistent; it seems that the precise value of trap effectiveness is not known (Galko et al., 2016).

The total solids (TS) content of spruce bark beetle partial samples ranged from 35 to 92 wt% with an average value of 49.4 wt%. Bednarz and Kacprzyk (2012) report an average fresh live mass TS content of 36.2 wt%. These authors give a bulk density of fresh beetles of 70.0 kg m⁻³. The bulk density of our mixed sample was measured indicatively in a 2-L measuring cylinder; a value of 220 kg m⁻³ was determined. The difference was due, for example, to moisture of the sample and admixture of bark residues. Depending on the humidity and quantity, the bulk density will probably generally range between 200–250 kg m⁻³. The average loss on ignition (VS_{TS}) was very high at 87.8% TS. The gross calorific value was 21.35 MJ kg⁻¹ TS, comparable to that of coniferous wood. Because of high nitrogen content of 10.8% TS the C: N ratio was very low at 4.4. The sulfur content of 0.4% TS was comparable to phytomass. The total solids' elemental composition was used to calculate the summary formula on C₃₄₄H₅₄₉O₁₂₀N₆₇S, and to calculate the theoretical biogas and methane production according to Buswell's formula as modified by Richards (Richards et al., 1991) (released NH₃ is retained in solution and immediately buffered with bicarbonate formed from CO₂). The substrate had the theoretical biogas and methane production of 0.901 Nm³ kg⁻¹ TS and 0.485 Nm³ kg⁻¹ TS, respectively. In the BMP batch digestion test in the substrate reactors in the first days, the H₂ and H₂S contents in the biogas reached a harmless 130 ppm and 900 ppm respectively and

Table 3. The list of methods and standards used for measuring parameters inoculum, spruce bark beetle biomass, and anaerobic digestate.

Parameter	Method and standard
VFA/TIC	of pH 5.0 and 4.4 according to Prof. Weiland's method (Lossie)
pH-H ₂ O	Potentiometric analysis, EN 15933:2012
Total solids (105 °C)	Gravimetric analysis (drying), EN 15934:2012
Volatile solids (550 °C)	Gravimetric analysis (loss on ignition), EN 15935:2012
Total combustibles	Calculation, ASTM D7582-15
Volatile combustibles	TGA analysis, 800 °C, N ₂ atmosphere, ASTM D7582-15
Fixed carbon	TGA analysis, O ₂ atmosphere, ASTM D7582-15
Ash	TGA analysis, O ₂ atmosphere, ASTM D7582-15
Lipids	Total lipids after hydrolysis, extraction, CSN 467092-7
Simple carbohydrates	Sugars extracted by 40% EtOH, hydrolysis, reduction by Luff-Schoorl reagent, CSN 46 7092-22
Starch	Ewers method, hydrolysis, polarimetry, CSN 560512-16
Crude fiber	Mergenthaler method, mineralization, ISO 6865:2000
Crude protein	Crude protein, CSN 467092-27, 6.25 ' total nitrogen according to Kjeldahl
Nitrogen-free extract	Calculated up to 100% of volatile solids (VS) content, CSN 46 7092-24
Nitrogenous substances	6.25 ' total nitrogen according to Kjeldahl method, ISO 5983:1997
Carbon	Combustion, CO ₂ determination, EN 15104:2011
Hydrogen	Combustion, H ₂ O determination, EN 15104:2011
Nitrogen	Combustion, NO ₂ determination, EN 15104:2011
Sulfur	Combustion, SO ₃ determination, EN 15289:2011
Oxygen	Combustion, imputation for O ₂ , EN 15104:2011
Ammonia nitrogen	Conway method with formaldehyde and titration, CEN/TS 16177
Nitrate nitrogen	Spectrophotometric analysis, CEN/TS 16177, UV-VIS, ISO 11905-1:1997
Volatile fatty acids (titration to pH 5.0)	Titration to 2 end points according to Prof. Weiland's method (HACH Lange TIM BIOGAS V02.2 titration station manual)
Total inorganic carbonate (titration, pH 5.0–4.4)	
Process stability parameter	
Volatile fatty acids C2–C5 (24 hours after sampling)	Capillary isotachopheresis, Villa Labeco application note
Ammonia nitrogen	Conway method with formaldehyde and titration, CSN P CEN/TS 16177
Nitrate nitrogen	Spectrophotometric analysis, CSN P CEN/TS 16177
Phosphorus	Photometric analysis, EN 14672:2005
Ca, K, Mg, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn, Se, As, Mo	Acid digestion, AAS analysis, EN 16174:2012
Mercury	AAS amalgam technique AMA 254 according to CSN 757440
Gross calorific value	Combustion, ISO 1928:2009
Enterococci	Cultivation at 37 °C, counting colonies, ISO 7899-2:1984, according to AHM 7/2001
Thermotolerant coliforms	Cultivation at 44 °C, CSN ISO 4832, CSN 75 7835, according to AHM 7/2001, AHM 1/2008
Salmonella	Proof test, CEN/TR 15215-1,2,3:2006, ISO 19250:2010, according to AHM 7/2001, AHM 1/2008
<i>Listeria monocytogenes</i> (25 g sample)	Cultivation at 37 °C, counting colonies, modified EN ISO 11290-1:1999
Yeasts and molds	Cultivation at 25 °C, counting colonies, ISO 21527-1.2
Thermophilic microorganisms	Cultivation at 55 °C, counting colonies, EN ISO 8199:2007
Mesophilic bacteria	Cultivation at 37 °C, counting colonies, EN ISO 6222:1999
Psychrophilic bacteria	Cultivation at 20 °C, counting colonies, EN ISO 6222:1999

Table 4. The catches of spruce bark beetles and numbers of pheromone traps in the years 2005–2018 in Tatra National Park.

Year	Total number of catches (1 ' 10 ⁶)		Average number of catches per pheromone trap		Number of pheromone traps	
	IT	PC	IT	PC	IT	PC
*2004	-	-	-	-	-	-
2005	21.7	4.2	8 574	13 524	2 528	313
2006	110.6	104.8	22 491	90 089	4 919	1 063
2007	110.2	121.8	22 666	116 194	4 864	1 048
2008	93.2	40.5	22 230	35 092	4 194	1 153
2009	111.4	57.6	28 443	49 106	3 918	1 173
2010	67.2	1.4	19 578	14 523	3 436	97
2011	18.2	1.7	18 131	12 760	1 002	132
2012	11.9	4.4	15 538	44 355	770	99
2013	9.7	3.1	12 405	34 615	785	90
**2014	2.9	0.81	4 280	11 096	681	73
2015	7.1	2.4	11 648	48 733	605	49
2016	9.9	0.99	16 983	19 947	585	50
2017	19.3	1	33 766	21 358	570	48
2018	16.2	1.2	28 189	27 965	576	43
Average	39.8	24.71	18 923	38 526	-	-
SE	11.79	11.07	2183.46	8233.29	-	-
Total	477.6	345.9	264 922	539 357	-	-

* windstorm “Alžbeta 19.11.2004”, ** windstorm “Žofia 15.5.2014”; IT: *Ips typographus* L., PC: *Pityogenes chalcographus* L.

then declined quickly. There was probably no significant inhibition of methanogenesis due to H₂S content, but inhibition due to N-NH₄ and free ammonia could occur. In all reactors, the CH₄ content exceeded 65 vol.% within the first 10 days and continued to rise slightly. The 40-day increase in biogas volume was already relatively low (Figure 3). The specific biogas and methane production of the substrate within 40 days reached 0.328 Nm³ kg⁻¹ TS and 0.242 Nm³ kg⁻¹ TS, respectively. This means the yield to theory of biogas and methane reached 36.4 % and 49.9%, respectively. For comparison, the average yield to theory of biogas and methane from 40 tests of different samples performed at the workplace, especially on phytomass such as maize silage or biowaste such as from the maintenance of urban greenspace, reached 53.4% and 57.5%, respectively. The most common biogas substrate—maize silage, according to the authors' experience, gave the biogas and methane production of 0.551 Nm³ kgTS⁻¹ and 0.287 Nm³ kg⁻¹ TS, respectively. Zauner and Kunzel (1986) achieved methane production of 0.270–0.289 Nm³ kg⁻¹ TS from anaerobic processing of maize silage in a laboratory batch

reactor. Mayer et al. (2013) reported on a set of 50 maize silage samples with average biochemical methane production of 0.304 Nm³ kg⁻¹ TS. From this, it can be estimated that the methane yield from the spruce bark beetles sample was measured correctly. Obviously, even grinding into small particles did not ensure the decomposition of chitin and other substances from the bark beetles' cuticles.

In the fed-batch digestion test, the organic loading was set to approximately 1.4 kg VS m⁻³ day⁻¹ until day 35, then it was increased to approximately 1.9 kg VS m⁻³ day⁻¹ (Figure 4). The H₂ content in biogas was relatively stable throughout the test and did not exceed 100 ppm, suggesting that methanogens were not overloaded. The VFA/TIC ratio in the batch rose from 0.24 to 0.38, meaning that organic acids had not started to accumulate above the healthy level, but it could have happened relatively soon. The H₂S content also increased continuously and reached 1600 ppm on day 52 day of the test (day 60 if the inoculum startup phase has been counted). In the next period, strong inhibition by H₂S would definitely occur. The daily biogas production reached 0.006 Nm³ d⁻¹ (Figure

Figure 3. Substrate biogas and CH₄ production in the BMP test.

Figure 4. Fed-batch process loading, VFA/TIC ratio, and H₂ and H₂S contents in raw biogas.

5). Linearly interpolated total solids content of the batch increased from 5 to 8 wt%. The CH₄ content reached 68 vol.% between days 25 and 40, then started to decrease slowly to 60 vol.%. Within 52 days, the average specific biogas and methane production reached 0.218 Nm³ kg⁻¹ TS and 0.140 Nm³ kg⁻¹ TS, respectively. This means that the yield to theory of biogas and methane was only 24.2% and 28.9%, respectively. The protective cuticles of bark beetles were not significantly degraded. These were distinct in the digestate upper layer (Figure 6). The digestate was

quite rich in nitrogen (6.09% TS), but the other element contents did not differ too much from fertilizer made from manure or phytomass. The gross calorific value of the digestate solids was not much higher than the value of the inoculum solids. The figure shows an increase in the solid fraction of the high-solids digestate. As expected, despite drum rotation, the beetles formed a crust at the level of the batch. The final solids content had to be determined after homogenization of the digestate using a laboratory mixer under a N₂ atmosphere.

Figure 5. Fed-batch process total solids, biogas production, and CH₄ content.

Figure 6. The final solids from the fed-batch digestion test in the Terrafor IS reactor.

At the average organic loading rate of 1.019 kg VS m⁻³ day⁻¹, the H₂ content in the biogas never exceeded 80 ppm, indicating stable methanogenesis. The VFA/TIC ratio increased only within a safe interval. The H₂S contents in biogas reached 800 ppm quickly and further increased to 1500 ppm. This could be one of the reasons for a reduction in biogas production after day 40 of the test. The main reason could be the accumulation of N-NH₄ and free ammonia.

Since the “Alžbeta” windstorm (in November 2004), the populations of spruce bark beetles have been intensively monitored with pheromone traps in Tatra National Park (Slovakia). At the same time, damage caused by the pests has been estimated annually. There were significant differences between the years in numbers of spruce bark

beetles captured; the occurrence of spruce bark beetles was highest in the third and fourth year after the windstorm. In addition to pest monitoring by pheromone traps in forests, this produces biological waste which must be eliminated. The mass of dead spruce bark beetles collected from pheromone traps is nitrogen-rich biomass coprocessable at biogas plants. The discontinuous mesophilic anaerobic digestion test showed a needed decomposition time of more than 40 days. The specific biogas and methane production were in line with high expectations. The single-stage fed-batch mesophilic digestion process was feasible at the mean organic loading rate of 1.0 kg VS m⁻³ day⁻¹, which was lower than the typical continuous wet process loading at agricultural biogas plants. Higher loading should be feasible during codigestion with carbon-rich phytomass. The inhibition by hydrogen sulphide was not significant during the 52 days of the test, but inhibition by ammonia seemed to take place. The digestate was inhomogeneous due to nondecomposed cuticles of beetles. Data can be a guide if, for example, a development of biorefinery using insects as a source of biomass is considered.

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