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Steam pretreatment of spruce forest residues: Optimal conditions for biogas production and enzymatic hydrolysis

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ABSTRACT

Steam refining of non-debarked spruce forest residues was investigated as pretreatment for enzymatic hydrolysis as well as for biogas production. Pretreatment conditions were varied in the range of 190–220 °C, 5–10 min and 0–3.7% SO_2 according to a statistical design. For both applications highest product yields were predicted at 220 °C and 2.4% SO_2, whereas the reaction time had only a minor influence. The conformity of the model results allows the conclusion that enzymatic hydrolysis is a suitable test method to evaluate the degradability of lignocellulosic biomass in the biogas process. In control experiments under optimal conditions the results of the model were verified. The yield of total monomeric carbohydrates after enzymatic hydrolysis was equivalent to 55% of all theoretically available polysaccharides. The corresponding biogas yield from the pretreated wood amounted to 304 mL/g_{ODM}. Furthermore, furans produced under optimal process conditions showed no inhibitory effect on biogas production.

It can be concluded that steam refining opens the structure of wood, thus improving the enzymatic hydrolysis of the polysaccharides to fermentable monomeric sugars and subsequently enabling a higher and faster production of biogas. Anaerobic fermentation of pretreated wood is a serious alternative to alcoholic fermentation especially when low quality wood grades and residues are used. Anaerobic digestion should be further investigated in order to diversify the biorefinery options for lignocellulosic materials.

1. Introduction

Today, there is no dispute that the use of renewable energy has to increase in order to reduce the CO_2 net emissions and the dependence on finite fossil fuels. Consequently, in 2008 the EU passed a directive in order to enhance the contribution of renewable energies (such as wind power, hydro power, solar energy, geothermal energy and biomass) to 20% of the total energy needs by 2020. Additionally, a 10% use of green fuels in transport is included within the overall EU objective (European Commission, 2008). The share of renewable energy sources in Europe's primary energy consumption increased from 4.4% in 1990 to 9.5% in 2010 (Eurostat, 2012). Thus, to achieve the 20% share of renewable energy in 2020 significant efforts will still be needed in the future. In Europe, 70% of the energy from renewable resources is gained by the use of biomass. One considerable advantage of biomass compared to the other renewable energy sources is the fact that dried biomass or biobased liquid fuels can be stored and thus can be utilised as and when they are needed. Currently the production of bioenergy from wood is mainly based on incineration, while biofuels or chemical products from biomass are mainly based on annual plants like corn, wheat or sugar beet. In order to reduce the raw material competition for biomass in energetic, material, and food applications it is desirable to increase the input of residual woody biomass to obtain energy or chemicals.

Conversion processes based on fermentation require the hydrolysis of cellulose and hemicelluloses into their corresponding monomeric carbohydrates in order to make them accessible for microorganisms. However, in case of lignocellulosic biomass the matrix of lignin and hemicelluloses around the cellulose fibrils, the low pore volume of the cell walls, and the high crystallinity of the cellulose fibrils limits the rate and extent of the enzymatic hydrolysis (Chang & Holtzapple, 2000). Therefore, different physical, chemical or physicochemical pretreatment methods such as mechanical comminution (chipping, grinding, milling), steam

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explosion, organosolv pulping, alkaline hydrolysis as well as diluted and concentrated acid hydrolysis have been investigated to enhance the enzymatic hydrolysis of lignocellulosic materials (Galbe & Zacchi, 2007; Hendriks & Zeeman, 2009; Kumar, Barrett, Delwiche, & Stroeve, 2009; Taherzadeh & Karimi, 2008). Besides dilute acid hydrolysis, steaming (steam explosion or steam refining) with and without addition of acid catalyst is the most investigated pretreatment method to enhance the accessibility of lignocellulosic materials for subsequent biological degradation processes (Galbe & Zacchi, 2002; McMillan, 1994). In case of wood the steam pretreatment is viewed as a cost effective process with a simple set up and low need for chemicals (Clark & Mackie, 1987; Jorgensen, Kristensen, & Felby, 2007; Sun & Cheng, 2002). In the steam explosion method biomass is treated under high pressure with saturated steam. Then the pressure is suddenly reduced, which makes the material undergo an explosive decompression. However, some investigations have shown that the explosion step is not necessary in order to increase the digestibility of wood (Brownell & Saddler, 1987; Brownell, Yu, & Saddler, 1986). Refining of the steamed material in a laboratory defibrator is a possible alternative to the explosion step. Schütt, Westereng, Horn, Puls, & Saake (2012) compared steam refining with steam explosion using the same raw material, namely not debarked poplar, and the same steam conditions. Regardless of the method used for the pretreatment, the results from enzymatic hydrolysis showed no significant difference concerning the carbohydrate yields and the overall product balance.

In the present study steam refining was tested as pretreatment for the conversion of spruce forest residues into biogas. The steam refining conditions time, temperature and SO_2-charge were optimized by an experimental design based on the response surface methodology (Box, Hunter, & Hunter, 1978). Since debarking of forestry residues is technically difficult and uneconomic the spruce wood was used without debarking in this investigation. The effectiveness of steam refining was assessed by biogas experiments as well as by conducting separate enzymatic hydrolysis tests in order to determine the accessible carbohydrate yields. In doing so, it should be evaluated if the separate enzymatic hydrolysis can be used as a fast test method in order to evaluate the degradability of pretreated lignocelluloses in the time consuming biogas process. Furthermore, furfural and 5-hydroxymethylfurfural (5-HMF) contents of the extracts after steaming were determined and their inhibitory influence on the biogas process was investigated.

2. Materials and methods

2.1. Raw material

Spruce forest residues originate from Reinbek Forest near Hamburg (Germany). The trunks and branches with diameters between 2 and 15 cm were chipped with their adherent bark in a shredder typical for landscaping applications. The shredded material used for steam refining experiments showed an inhomogeneous particle size from 10 to 60 mm in length and from 5 to 15 mm in thickness and contained about 8% bark. The wet chips were stored in a cooling chamber and were defrosted one day before the steam experiments.

2.2. Analytical methods for raw material characterisation

The extractives of the wood and bark fractions were analyzed by accelerated solvent extraction (ASE 200, Fa. Dionex). Therefore, about 1 g of milled sample materials were extracted successively by petrol ether, acetone:water (9:1) and water at 100 bar and 100 °C for 40 min. The total extractive content was determined by adding the single extraction yields. Ash contents were determined by combustion at 525 °C (Tappi Standard - T211om-93, 1993).

Carbohydrate and lignin contents were analyzed by a two-stage acid hydrolysis. 200 mg of dry material were hydrolyzed with 2 mL of 72% H_2SO_4 at 30 °C for exactly 1 h to break up the crystalline structure of the cellulose. The samples were then diluted with water to a concentration of 4% H_2SO_4 and put in an autoclave for 40 min at 120 °C for the hydrolysis to monomeric sugars. After cooling, the samples were filtered on a No. 4 sintered glass crucible. The residue was weighed on an analytical balance (AG 245, Mettler Toledo) and represents the acid-insoluble lignin content. The carbohydrate content in the hydrolysates was analyzed by borate-anion-exchange-chromatography with after-column derivatization and detection of the separated monosaccarides at 560 nm as described previously (Sinner & Puls, 1978; Sinner, Simatupang, & Dietrichs, 1975; Willfor et al., 2009).

2.3. Steam refining pretreatment

Steam refining was performed in a 10 L reactor. Material input per batch was 300 g_{DM} of wet spruce chips and the reactor was completely filled with saturated steam.

Treatments were performed at 190–220 °C for 5–10 min with a SO_2-charge of 0–3.7% based on dry wood. In total, 25 steam refining experiments were conducted for the optimization of the three process parameters. The addition of SO_2 was carried out prior to the steam pretreatments. The wood was put into a plastic bag and the required SO_2 amount was fed into the bag. Finally, the SO_2 uptake was controlled on a balance.

The reactor contained inside a blade system, which was rotated at 1455 rpm for 30 s at the end of each steam treatment, to defibrate the softened material. By flashing with additional water the reactor was emptied and a washing of the pretreated material occurred. The extract formed during steam treatment and the wash water was separated from the fibers in a spin dryer. This extract contains the water soluble compounds (monomeric or oligomeric sugars, furans and organic acids) after steaming. Yields of the steam pretreated solid fibers and of the liquid extract were determined after drying.

2.4. Application tests of steam pretreated samples

The effectiveness of the steam refining pretreatment was evaluated by the total monomeric carbohydrate yield after hydrolysis of the fiber and extract fraction. Additionally, the produced biogas volume after anaerobic fermentation of the combined fiber and extract fractions was measured.

2.4.1. Total monomeric carbohydrate yield after hydrolysis

The above mentioned total monomeric carbohydrate yields after hydrolysis of the pretreated samples were calculated by the sum of carbohydrates yields after enzymatic hydrolysis of the fiber fraction and after acid hydrolysis of the extract fraction. Preliminary experiments with extracts showed that the monomeric carbohydrate yields of enzymatic hydrolysis are very close to those of acid hydrolysis. Furthermore, HPLC–MS measurements revealed that the hemicellulose degradation products exist as monomers or oligomers in the extract after steam refining. Therefore, it can be assumed that all carbohydrates in the extract are easy to degrade by enzymatic hydrolysis and it is permissible to determine the monomeric yields of the extract directly by acid hydrolysis.

Hydrolysis of the extracts was carried out after freeze-drying. Lyophilized samples (100 mg) were dissolved in 10 mL water, spiked with 1.8 mL of 2 N H_2SO_4 (1.5% H_2SO_4) and autoclaved at 120 °C for 40 min.

Enzymatic hydrolysis of the fibers was conducted with 21 FPU (filter paper units) Celluclast 1.5 L (Cellulase complex, Novozymes

A/S) and an addition of 50 μL Novozym 188 (β-glucosidase, Novozymes A/S) per g substrate. All experiments were carried out at 4% dry matter content in phosphate citrate buffer pH 4.8 for 72 h at 45 °C. Filter paper activity of celluclast was determined according to the NREL laboratory analytical procedure (Adney & Baker, 1996).

2.4.2. Gas formation potential within 21 days (GF_{21})

Prior to the GF_{21}-tests (Verein Deutscher Ingenieure, 2006) fiber and extract fractions were combined according to their yields ratio after each steam refining experiment. The used inoculum was delivered by a municipal sewage plant. Due to their nature, sludges of this kind come into contact with a large variety of substances and thus constitute an inoculum which contains a diversified biocoenose. The activity of the inoculum was tested in reference tests using microcrystalline cellulose (Avicel) as substrate. By this procedure a biogas production of about 670 mL/g_{ODM} was measured. According to the molecular formula of glucose ($C_6H_{12}O_6$) and by the use of the equation after Buswell and Müller (1952) the fermentation of 1 g_{ODM} Avicel results in a theoretical biogas yield of about 750 mL. Thus, the used inoculum produced 89% of the theoretically possible biogas yield from Avicel. According to the guidelines of the Verein Deutscher Ingenieure (2006) the activity of the inoculum shows an adequate level if at least 80% of the theoretically possible biogas production could be reached.

The biogas formation potential from the combined samples was determined volumetrically by using eudiometers at 35 °C. For this purpose the steam pretreated fractions (substrate) were kept in 500 mL glass vessels together with the inoculum (sewage sludge), whereby the ratio of organic dry matter of substrate to inoculum was adjusted to 0.5. For a period of 21 days, produced biogas volumes were determined every day.

Furthermore, the inhibitory effect of furans on the biogas production was tested by using Avicel as a model substrate. For this purpose the biogas production from Avicel was measured after the addition of 2.5 mg furfural and 15 or 30.0 mg 5-HMF per 1 g Avicel substrate and compared to biogas production from pure Avicel. All experiments in combination with the inhibitory effect of furans were also conducted to the above mentioned guidelines of the GF_{21}-tests.

2.5. Analytical methods

The carbohydrate content in the hydrolysates was analyzed by borate-anion-exchange-chromatography with after-column derivatization and detection at 560 nm as described previously (Sinner et al., 1975; Sinner & Puls, 1978; Willfor et al., 2009).

Furfural and 5-HMF contents of the extracts were analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) with separation on an Aquasil C18 column (Thermo Scientific,Waltham, MA, USA) and UV detection at 210 nm.

2.6. Statistical model

The effects of temperature, time and SO_2-charge of the steam-pretreatment were evaluated based on the response surface methodology (Box et al., 1978). For this purpose, a statistical experimental design with 25 experiments was planned, using the JMP software by SAS. The boarders of the studies effects (temperature 190–220 C, time 5–10 min, SO_2-charge 0–3.7%) were fixed on the basis of earlier investigations. The following equation shows the general prediction formula, where Y is the response target, while temperature (T), time (t) and SO_2-charge (SO_2) are the parameters and a_0, a_i, a_{ij} are the estimated coefficients of regression.

$$Y = a_0 + a_1T + a_2t + a_3SO_2 + a_4Tt + a_5TSO_2 + a_6tSO_2 + a_{11}T^2 + a_{21}t^2 + a_{31}SO_2^2$$

The parameters were scaled and centered, so that the highest value corresponded to 1 and the lowest to −1. To fit the formula to the experimental results, a backward stepwise regression was used, following a given scheme within the statistical software (SAS Institute Inc., 2010). During this procedure, the least significant terms were removed stepwise from the model and the reduced equation calculated again until all remaining terms were significant with a significance level ≥ 90% (p-value ≤ 0.1). Terms that were not significant at this level, but were included in another term that was significant were also kept in the equation (e.g. the factor time is not deleted from the equation, if the factor temp * time is significant). After creating a prediction formula for each response target, results could be predicted depending on temperature, time and SO_2-charge of the treatment.

3. Results and discussion

3.1. Raw material characterization

All experiments were conducted with spruce forest residues containing a bark proportion of 8%. The wood was utilized with bark in order to include possible inhibitory effects of the extractive rich bark proportion on steam treatment, enzymatic hydrolysis and biogas production. Table 1 shows the raw material characterization for pure wood and bark proportion, as well as the resulting values for the total raw material. Compared to the bark proportion the wood proportion contains a higher carbohydrate content of 67%, whereas the lignin and extractive content are lower. Calculated from the pure bark and wood proportions the total raw material shows a lignin content of about 30% and a carbohydrate content of 66%. The carbohydrate content representing the fermentable part of the raw material, whereas lignin is not fermentable. It has to be mentioned that the hydrolysis residue which is denominated as “lignin” is contaminated by some bark components e.g. waxes. However, this small systematic error is acceptable, since this material is not fermentable as well, and the bark is by far the smaller proportion of the raw material.

3.2. Statistical model data

The optimization of steam refining for subsequent enzymatic hydrolysis or biogas production is based upon a factorial design of 25 experiments. Table 2 represents the process parameters temperature (190–220 °C), time (5–10 min) and the SO_2-charge (0–3.7% based on raw material) for each steam refining experiment and lists the corresponding results for fiber, extract, total carbohydrate and biogas yields. Furthermore, the furfural and 5-HMF concentrations in the produced extracts are shown in Table 2. Based on these results for each response (fiber, extract, total carbohydrate, biogas, furfural and 5-HMF) a prediction formula was fitted as described in Section 2.6. In Table 3 the coefficient of determination (R^2) and the adjusted coefficient of determination (R^2 adj.) are presented for

Table 1
Chemical composition of spruce raw material.

Substances	Wood	Bark	Total raw material
Ratio [%]	92	8	–
Cellulose [%]	41.6	34.1	41.0
Mannan [%]	16.2	7.1	15.5
Xylan [%]	9.0	8.7	9.0
$\sum$ Carbohydrates[a] [%]	67.0	50.4	65.7
Lignin [%]	29.5	33.6	29.8
Extractives [%]	3.6	18.0	4.7
Ash [%]	0.4	0.8	0.4

[a] $\sum$ Carbohydrates: sum of HPLC-detected glucose, xylose, mannose, arabinose, galactose and 4-O-methylglucuronic acid after 2-step acid hydrolysis.

Table 2
Experimental design of the steam refining series and selected results from subsequent investigations.

Run	Temp. [°C]	Time [min]	SO_2-charge [% wood][a]	Fiber yield [% wood]	Extract yield [% wood]	Acid hydro. extract [%]		Enzymatic hydro. fibers [%]		Furfural [mg/g_{wood}][b]	5-HMF [mg/g_{wood}]	Total monomeric carbohydrates [% wood]	Biogas [mL/g_{ODM}][c]
						Glc[d]	$\sum$[e]	Glc	$\sum$				
1	190	5	0	79.1	12.7	7.9	60.6	1.3	2.9	0.10	0.21	10.0	104
2	190	7.5	1.1	75.7	26.3	11.0	68.3	7.2	8.5	0.55	1.57	24.5	170
3	190	10	0	75.3	16.9	9.2	67.3	3.5	4.9	0.17	0.38	15.1	99
4	190	10	2.6	69.8	25.1	12.5	69.0	7.6	8.8	0.81	2.76	23.4	159
5	190	5	2.5	68.4	26.6	11.9	66.1	10.6	12.4	0.68	1.51	26.1	175
6	200	7.5	0	67.2	14.9	10.2	70.2	5.2	6.8	0.32	0.69	15.0	122
7	200	7.5	1.2	64.1	24.0	15.2	70.2	16.6	18.0	1.01	4.04	28.4	191
8	200	7.5	2.3	58.9	22.7	16.8	66.5	18.8	20.1	1.38	4.45	26.9	193
9	200	5	1.2	60.0	22.6	14.0	71.7	11.6	13.3	0.63	2.19	24.2	180
10	200	10	1.3	67.1	24.9	16.8	67.8	20.5	21.8	1.39	6.85	31.5	200
11	200	7.5	1.4	63.1	23.8	15.1	67.2	17.1	18.5	0.90	4.16	27.7	192
12	210	5	2.5	50.5	27.4	22.7	62.5	33.6	35.0	1.31	7.40	34.8	191
13	210	7.5	1.3	63.7	25.7	21.1	65.2	31.4	32.9	1.56	9.26	37.7	224
14	210	5	0	66.9	16.7	9.0	64.6	5.6	6.9	0.39	0.94	15.4	119
15	210	10	0	76.2	19.6	10.3	55.7	7.3	8.2	1.52	4.44	17.2	113
16	210	10	1.7	58.7	24.0	24.8	57.2	35.2	36.3	1.56	13.06	35.0	175
17	210	10	2.5	53.2	27.1	24.2	55.4	34.3	35.3	1.52	12.63	33.8	220
18	220	10	3.5	34.4	24.2	25.0	39.9	30.8	31.5	1.23	16.3	20.5	195
19	220	10	3.7	35.0	28.0	24.4	40.1	34.2	34.9	1.08	16.66	23.5	226
20	220	5	0	80.0	21.2	10.3	63.7	7.3	8.5	0.55	2.53	20.3	123
21	190	10	3.5	66.0	24.2	13.4	66.9	14.1	15.8	0.58	3.15	26.7	157
22	220	10	0	73.6	17.3	11.8	52.5	12.6	13.4	0.66	8.62	18.9	120
23	220	5	3.7	57.5	24.8	21.4	66.6	29.3	30.1	0.84	9.69	33.8	173
24	210	10	3.5	42.8	34.3	21.5	46.6	29.4	30.4	2.94	14.97	29.0	199
25	190	5	3.6	67.7	26.4	13.5	69.5	11.5	13.3	0.48	1.75	27.4	150

[a] % wood: % based on initially used spruce material (dry matter).
[b] mg/g_{wood}: mg furans in the extract per g (dry matter) initially used spruce material.
[c] mL/g_{ODM}: mL produced biogas per g (organic dry matter) of pretreated spruce substrate.
[d] Glc: glucose.
[e] $\sum$: Sum of all monomeric carbohydrates, detected in the hydrolyzates after acid or enzymatic hydrolysis.

Table 3
Fitted correlation coefficients and *p*-values of the prediction formulas for fiber, extract, total carbohydrates, biogas, furfural and 5-HMF yields.

Scaled factors	Fiber yield		Extract yield		Total carbohydrates		Biogas		Furfural		5-HMF	
	Estimate	*p*-Value	Estimate	*p*-Value	Estimate	*p*-Value		Estimate	*p*-Value	Estimate	*p*-Value	Estimate
Intercept	59.61	<0.0001	25.13	<0.0001	31.75	<0.0001	Intercept	59.61	<0.0001	25.13	<0.0001	31.75
Temp.	−6.40	<0.0001	–	–	3.18	0.0017	Temp.	−6.40	<0.0001	–	–	3.18
Time	−1.03	0.3013	–	–	−0.26	0.7221	Time	−1.03	0.3013	–	–	−0.26
SO_2	−10.85	<0.0001	5.10	<0.0001	5.53	<0.0001	SO_2	−10.85	<0.0001	5.10	<0.0001	5.53
Temp.^{2}	7.55	0.0010	–	–	–	–	Temp.^{2}	7.55	0.0010	–	–	–
Time^2	–	–	–	–	–	–	Time^2	–	–	–	–	–
$SO_2{}^2$	–	–	−3.53	0.0115	−11.33	<0.0001	$SO_2{}^2$	–	–	−3.53	0.0115	−11.33
Temp.*time	−2.93	0.0261	–	–	−1.92	0.0494	Temp.*time	−2.93	0.0261	–	–	−1.92
Temp.*SO_2	−5.57	0.0007	–	–	–	–	Temp.*SO_2	−5.57	0.0007	–	–	–
Time*SO_2	−3.02	0.0222	–	–	−2.46	0.0147	Time*SO_2	−3.02	0.0222	–	–	−2.46
R^2	0.922		0.705		0.851		R^2	0.922		0.705		0.851
R^2 adj.	0.891		0.678		0.810		R^2 adj.	0.891		0.678		0.810

each calculated prediction formula. R^2 adj. is useful to make models with different numbers of parameters more comparable. This goal is achieved by integrating the degrees of freedom of the model in its computation. The highest vales of R^2 adj. are given for the prediction formulas of the fiber yield and the 5-HMF concentration. The values of 0.89 and 0.95 signify very high correlations. For carbohydrate yields after hydrolysis and biogas yields R^2 adj. of 0.81 and 0.85 were calculated. The extract yield and the furfural concentration show the lowest values of below 0.7. Altogether, the coefficients of determinations can be considered as satisfactory, in view of the complexity of the experiments and the number of processing steps.

Furthermore, Table 3 gives the estimated coefficients of regression (estimates) together with their *p*-values for the analyzed process parameters (temperature, time and SO_2-charge). Basically, the higher the absolute values of the estimates, the larger are their impact on the examined response. For the fiber, extract, carbohydrate and biogas yield the SO_2-charge has a very high and significant influence compared to the parameters temperature and time. In contrast, the furfural and 5-HMF concentrations in the extracts are influenced mostly by the temperature. The parameter time has no influence on the extract and biogas yield. In the case of the fiber and carbohydrate yields the single parameter time has *p*-values higher than 0.1 and were kept in the model only because it is part of the interaction terms "temp*time" and "time*SO_2" (Table 3). It has to be emphasized that the impact of the reaction time is less important than the impact of temperature and SO_2. Therefore, the response surface diagrams in Figs. 1–3 are depicted based on the SO_2 input and temperature during the pretreatment and the time was fixed to 5 min for this representation.

3.3. Steam refining yields and furan concentration of the extracts

After steam refining two fractions are obtained. The liquid extract is made up mainly by hemicellulose sugars and some degradation products. The fiber fraction consists mainly of lignin and cellulose.

Fig. 1 depicts the response surface diagrams for fiber and extract yields after steam pretreatment. The main product after steaming is the fiber fraction. The fiber yields range between 50 and 80.0% (Fig. 1a), whereas the yield of the extract is between 18 and 26% of the initially used raw material (Fig. 1b). Under harsh steaming conditions the combined yield (fiber and extract) amounts to less than 80%. This material loss is partly due to the formation of furans and their degradation products which is accompanied by a weight loss due to dehydration reactions. Furthermore, volatile degradation products in the vapour phase are partly lost during the decompression of the reactor after steam refining. Finally, the numerous process steps (like emptying the reactor, washing the material) additionally contribute to the material loss. The fiber yields are affected by the SO_2 input and the temperature (Fig. 1a). Between 190 and 205 °C the fiber yield is continuously decreased with increasing temperature, whereas in this range the yield is only slightly influenced by SO_2. Surprisingly, above 205 °C the yield starts to increase again. This phenomenon will be further discussed later on. However, for a given temperature the increased addition of SO_2 always causes a continuous reduction of the fiber yield. The extract yields in Fig. 1b are mainly influenced by the SO_2-charge. Up to 2.5% SO_2 a steady increase of the extract yield occurs, while at higher charges the yield diminishes. In order to explain the response surfaces models for the extract and fiber yields the main degradation reactions during steam refining have to be considered. During steaming or hydrothermal treatments hemicelluloses are intensively hydrolyzed starting at 170 °C, whereas the release of monosacharides from cellulose does not occur below 210 °C (Bobleter, 1994; Galbe & Zacchi, 2002). Therefore, a complete removal of the hemicelluloses is achieved without severe celluloses losses. In the course of this process the liberated monosaccharides can be further degraded to furans. By dehydration of monosaccharides, furfural is formed from pentoses and 5-HMF from hexoses. Furfural has a high volatility and reactivity and 5-HMF can be further oxidized to formic and levulinic acids (Dunlop, 1948; Sanchez & Bautista, 1988; Ulbricht, Northup, & Thomas, 1984).

Compared to carbohydrates, lignin has a much greater stability during steam pretreatment (Li, Henriksson, & Gellerstedt, 2005). Although the lignin structure is altered and partly degraded, only small amounts of lignin are released from the wood. This is due to condensation reactions which occur between lignin degradation products and as well between lignin and furfural under harsh steaming treatments (Bobleter, 1994; Burtscher, Bobleter, Schwald, Concin, & Binder, 1987; Pecina, Burtscher, Bonn, & Bobleter, 1986).

Considering the forementioned reactions the maximum of the extract yield can be explained by the degradation of the dissolved carbohydrates to furans and further degradation products. When the main hemicellulose fractions are hydrolyzed the further degradation and condensation reactions are dominating and the extract yield declines. Furthermore, the high reactivity of furans with lignin and with their own degradation products result into lignin modification and into additional high molar mass condensation products, which are insoluble and remain in the fiber fraction. Accordingly, it can be postulated that at high temperature and low SO_2-charge the formentioned reactions are increasing the fiber yield as depicted in Fig. 1a. Increased addition of SO_2 enables a further hydrolytic degradation of the fibers even at high temperature levels.

As pointed out before the furans formed under harsh steaming conditions are predominantly accumulated in the extract. These compounds are toxic and can affect the enzymatic hydrolysis as well as the growth and metabolism of several microorganisms

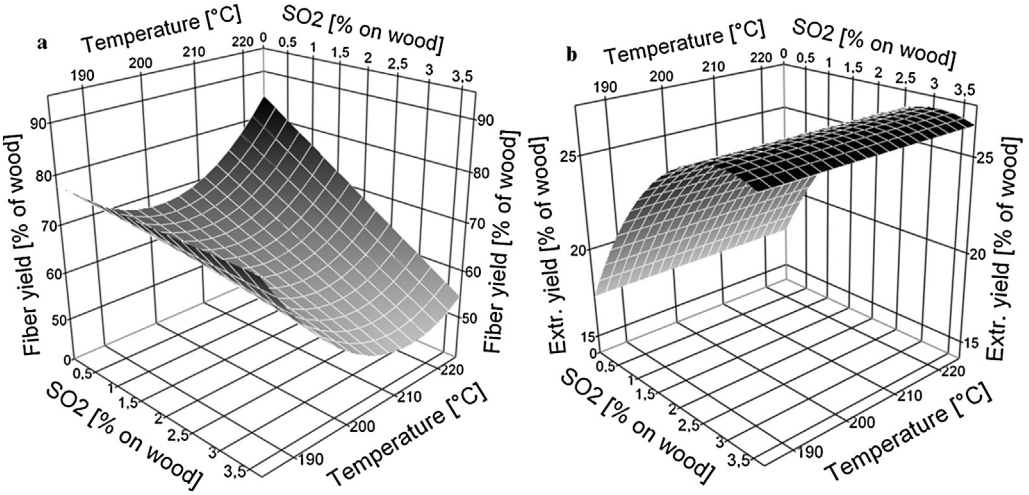

Fig. 1. Response surface models for fiber (a) and extract yields (b) after steam pretreatment.

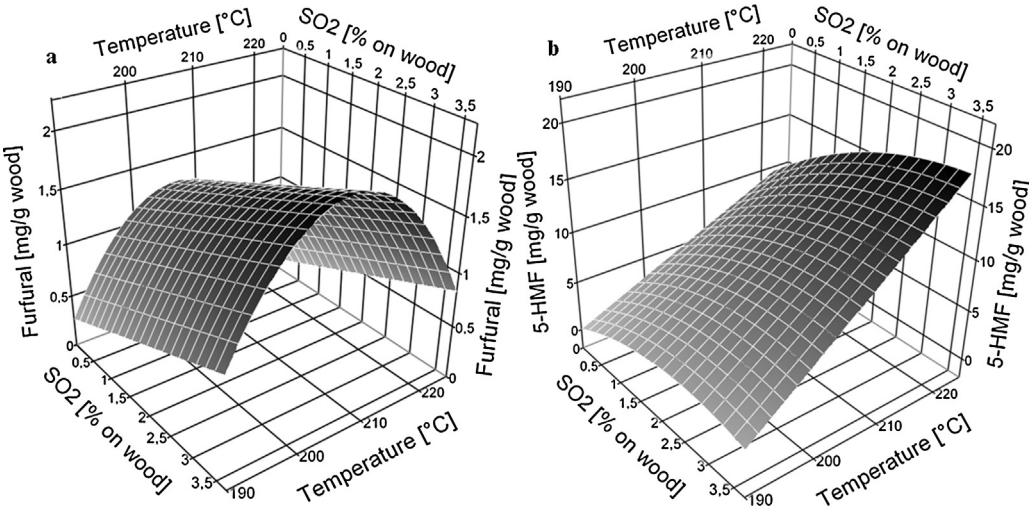

Fig. 2. Response surface models for furfural (a) and 5-HMF (b) formed in the extract during steam pretreatment.

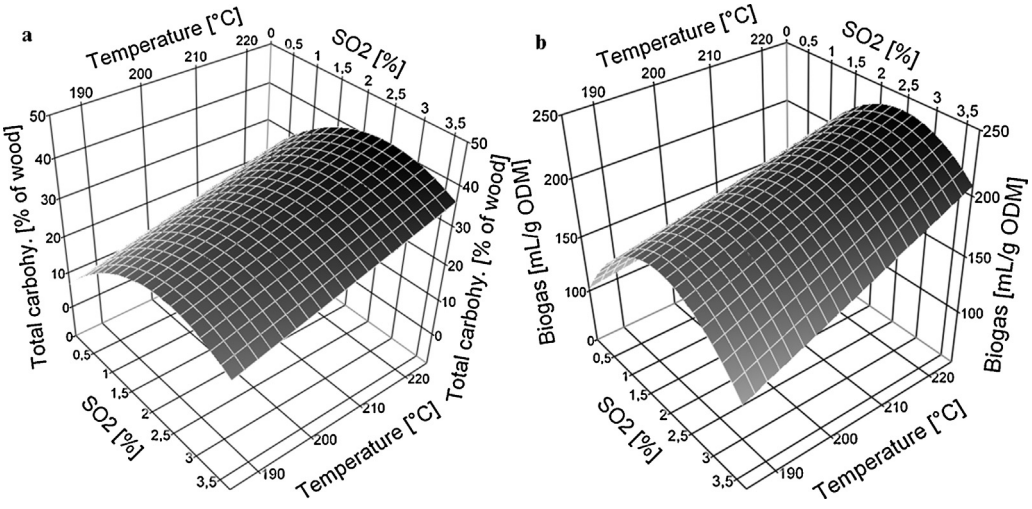

Fig. 3. Response surface models for total monomeric carbohydrate yield (a) after enzymatic hydrolysis and produced biogas volume (b).

Table 4
Comparison of the model prediction with three control tests (CT) conducted at the calculated optimal conditions (220 °C, 5 min, 2.4% SO_2).

	Fiber yield [% wood]	Extract yield [% wood]	Furfural [mg/g_{wood}]	5-HMF [mg/g_{wood}]	Total monomeric carbohydrate [% wood]
Model	60.9 ± 5.6	26.1 ± 1.3	0.68 ± 0.19	9.21 ± 1.65	39.3 ± 4.4
CT1	56.1	22.1	0.94	10.1	35.6
CT2	55.3	26.9	1.01	9.17	38.2
CT3	55.8	23.2	0.95	10.21	35.6
Average CT1-3	55.7 ± 0.4	24.1 ± 2.5	0.96 ± 0.04	9.82 ± 0.57	36.5 ± 1.5

(Kim, Ximenes, Mosier, & Ladisch, 2010; Klinke, Thomsen, & Ahring, 2004; Larsson et al., 1999). Therefore, the concentration of furans in the extracts was determined and included into the response surface model. The furfural (Fig. 2a) and 5-HMF concentrations (Fig. 2b) are most strongly influenced by the temperature and to a minor extend also by the SO_2-charge. The prediction model for furfural has only low correlation coefficients (R^2 = 0.708; R^2 adj. = 0.649; Table 3). This is at least partly the result of its high volatility which complicates a complete recovery from the reaction system. However, for the 5-HMF prediction model high correlation coefficients were determined indicating as well a reliable recovery and analysis of this component (R^2 = 0.963; R^2 adj. = 0.950; Table 3). For furfural the model predicts a maximum concentration of 2.02 mg/g_{wood} at 206 °C, 10 min and a SO_2-charge of 3.7%. The 5-HMF concentration is continuously increasing within the investigated temperature range. Thus, the highest 5-HMF concentration of 17.5 mg/g_{wood} was predicted for 220 °C, 10 min and a SO_2-charge of 3.7%. In comparison to furfural, the concentration of 5-HMF is much higher because 5-HMF is formed by hexoses which are derived from O-acetyl-galactoglucomannan, the main hemicellulose of softwoods. Under harsh process conditions 5-HMF can be produced additionally from glucose derived from cellulose hydrolysis. In contrast, furfural is generated solely from the arabinose and xylanose components of the arabino-4-O-methyl-glucoronoxylans, occurring in minor quantities of around 5–10% in softwoods (Timell, 1967; Sjöström, 1993).

3.4. Optimization of steam refining conditions for total monomeric carbohydrates yields after enzymatic hydrolysis and biogas production

The steam pretreated spruce was used for enzymatic hydrolysis and biogas tests (GF_{21}). After enzymatic hydrolysis the monomeric carbohydrate solution can be used for fermentation processes, while the non-degradable compounds such as lignin remain as insoluble residue. The enzymatic hydrolysis is a widely used lab method to test the effectivity of pretreatment processes for lignocellulosic materials. In contrast to that, the biogas test is a real end-use application test. The produced biogas contains 50–80% methane that can be used for the production of electricity, heat or fuel (Verein Deutscher Ingenieure, 2006; Rasi, 2009). The GF_{21} test determines the gas yield in a lab scale batch test within 21 days. The monomeric carbohydrate yields from enzymatic hydrolysis of the fibers and acid hydrolysis of the extracts were calculated based on raw material input and summed up for the development of the response surface model in Fig. 3a. According to the yield ratio after steam refining the GF_{21} tests were carried out with combined fiber and extract fractions. Fig. 3b depicts the response surface model of the produced biogas volume per gram organic dry matter (mL/g_{ODM}). The two response surface models show very similar effects of the analyzed process parameters. For the carbohydrate and the biogas yield the reaction time has a very small influence, while temperature and SO_2-charge are the key factors. It is apparent that the acid catalyst SO_2 has a stronger impact on total carbohydrate and biogas yield than the temperature in the investigated range. Schütt et al. (2013) compared the steam refining pretreatment of poplar with and without the addition of SO_2 by means of a statistical design. The investigation shows that the use of the acid catalyst SO_2 resulted in a decrease of the necessary treatment temperature. Thus condensation reactions and subsequent precipitation of lignin agglomerates on the fiber can be reduced if by the addition of SO_2 relative low temperatures could be applied within the investigated boundaries. Furthermore, the rising of the temperature results in a continuous increase of products, whereas the SO_2-charge exhibits a distinct maximum at about 2.4%.

The decrease of the total carbohydrate and biogas yields at SO_2-charges over 2.4% can be due to the degradation of monomeric carbohydrates of the extract. Under these harsh conditions also significant amounts of the cellulose are presumably degraded to 5-HMF. Furthermore, the formed degradation products like furfural and 5-HMF could inhibit the enzymatic hydrolysis or form non fermentable condensation products. The optimization calculation with the model equation gives the highest overall carbohydrate yield of 39.5% at 220 °C, 2.4% SO_2 and 5 min of steaming. The highest biogas production of 231 mL/g_{ODM} is also predicted after a pretreatment at 220 °C and 2.4% SO_2, in this case, however, the time has no significant influence. Under the predicted optimal conditions (220 °C, 5 min, 2.4% SO_2) three control experiments were conducted in order to verify the statistical model and the calculated optimum. Table 4 summarizes the model prediction and the experimental control data. The average results of fiber, extract and total carbohydrate yield are 3–5% below the predicted values, whereas the determined furfural and 5-HMF concentrations in the extract are 0.3–0.5 mg above the prediction. Thus, under optimal conditions, the experimentally determined total carbohydrate yield is 36.5% and the extracts contain 0.96 mg furfural and 9.82 mg 5-HMF per gram of dry raw material. Considering the complexity of the experiments and the number of processing steps, the predictability can be considered as satisfactorily.

3.5. Formation of biogas inhibitors under optimal steam refining conditions

The possible inhibitory effect of the furans on the biogas production was tested by the use of Avicel (microcrystalline cellulose) as a model substrate. Fig. 4a presents the biogas production of Avicel with and without addition of furfural and 5-HMF. The addition of 2.5 mg furfural and 15 or 30.0 mg 5-HMF per 1 g Avicel substrate was derived from the determined maximum values of 2.02 mg/g_{wood} furfural and 17.5 mg/g_{wood} 5-HMF contained in the extract after steam refining (see Section 3.3). The fermentation of the pure Avicel produces the highest biogas volume of 730 mL/g_{ODM}. The addition of 2.5 mg furfural in combination with 15 mg 5-HMF reduces the produced biogas volume only slightly to 705 mL/g_{ODM}. The further increasing of the furan addition to 2.5 mg furfural and 30 mg 5-HMF decreases the biogas volume to the lowest value of 670 mL/g_{ODM}. Thus, the results indicate that the addition of these high amounts of furans reduces the biogas production up to 10%. It should be emphasized that under the optimal process conditions only 0.96 mg/g_{wood} furfural and 9.82 mg/g_{wood} are produced. Accordingly, it can be postulated that under these

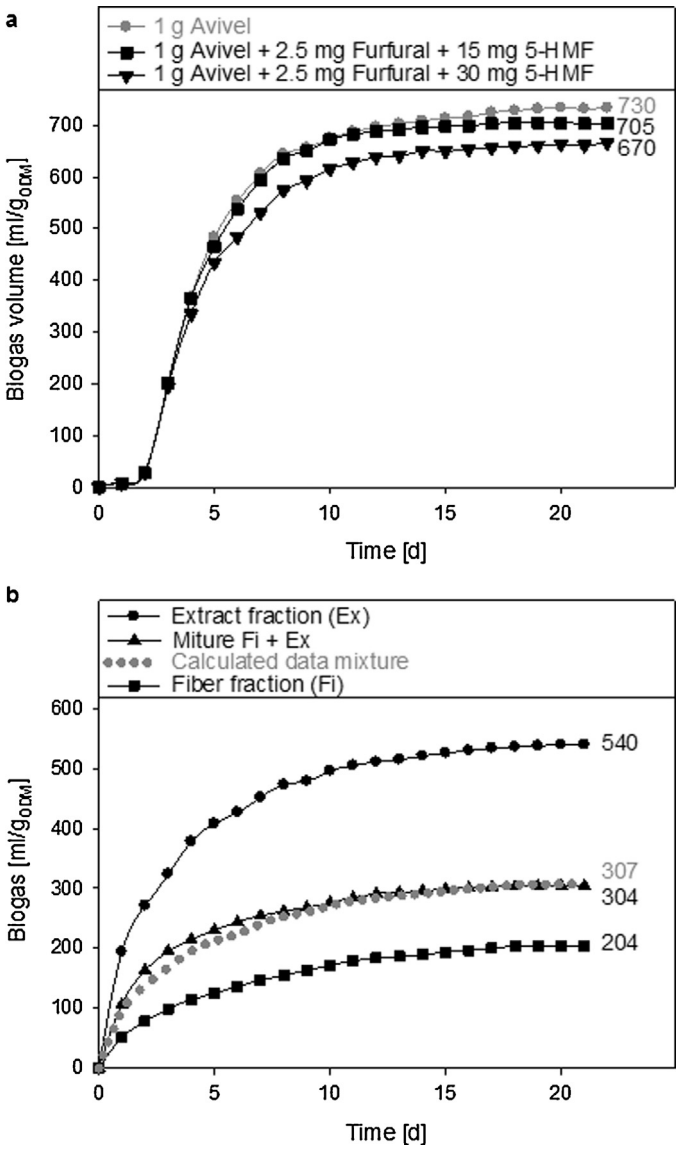

Fig. 4. Biogas production from Avicel with the addition of furfural and 5-HMF (a), as well as from steam pretreated (220 °C, 5 min, 2.4% SO_2) spruce fractions and their combined mixture (b).

pretreatment conditions nearly no inhibitory effect is caused by the furans.

In parallel to the effect of the furans on cellulose digestion the inhibitory influence of the fiber and extract fraction to each other was tested directly. For this case fibers and extract, obtained from steaming under optimal process conditions (220 °C, 5 min, 2.4% SO_2), were fermented separately as well as in a joined biogas test. The mixtures of fiber and extract fraction were mixed in the weight ratio of their production in the steam treatment. The biogas volumes of this test series are depicted in Fig. 4b.

The extract produced a high biogas volume of 540 mL/g_{ODM}. The fibers were less susceptible and produced only 200 mL/g_{ODM}. This difference could be explained by the high amount of easily fermentable monomeric and oligomeric carbohydrates in the extract, whereas the fiber fraction contained 50% of lignin which is enriched in the fibre fraction during the steam treatment. If the mixture of extract and fibers are jointly fermented the biogas production for the combined fractions amounts to 304 mL/g_{ODM}. Accordingly the joint fermentation of extract and fibers shows the same biogas production, which could be calculated from the individual fermentation of extract and fibers considering the yield proportion of both fractions (dotted grey line in Fig. 4b). This indicates that both fractions do not contain any inhibitive compounds, which might impede the activity of the microorganisms.

According to the surface response model, a maximum biogas production of 231 mL/g_{ODM} was predicted under optimal process conditions (see Section 3.4). Thus, in this test series the produced biogas volume of the joint fermentation of fiber and extract is about 70 mL/g_{ODM} higher. This difference is possibly related to various microorganism activities in the used inocula. Generally, the activity is influenced by the types and number of microorganisms in the inoculum. Thus, the activity has no constant value, but is depending on the history of the inoculum such as origin, storage time or last used substrate.

4. Conclusions

For highest total monomeric carbohydrate yields after enzymatic hydrolysis as well as for highest biogas yields, steam pretreatment conditions of 220 °C and 2.4% SO_2-charge were predicted by the statistical model. In contrast to the key factors SO_2-charge and temperature, the reaction time had only a minor influence. The conformity of the model prediction for enzymatic hydrolysis and anaerobic digestions indicates that the enzymatic hydrolysis is a suitable test method to evaluate the degradability of pretreated lignocellulosics in the biogas process.

Based on the model equations, a total carbohydrate yield of 39.3% (based on starting material) and a biogas production of 231 mL/g_{ODM} were calculated under optimal conditions. The predicted results were verified by control tests, resulting in a total carbohydrate yield of 36.5% and a biogas production of 304 mL/g_{ODM}. In view of the complexity of the experiment, such as working with living microorganisms, and the number of processing steps the model can be considered as satisfactory. In order to improve the precision of the prediction model, a future design should be built in the proximity of the determined optimum.

The total carbohydrate yield after enzymatic hydrolysis is equivalent to 55% of the theoretical available polysaccharides. (see Section 3.1, Table 1) The corresponding conversion rates in the literature are up to 90% for agriculture residues (e.g. corn stover), 65–80% for hardwoods (e.g. poplar and willow), and about 65% for softwoods (e.g. spruce and pine) (Galbe & Zacchi, 2007; Öhgren, Bura, Saddler, & Zacchi, 2007; Sassner, Martensson, Galbe, & Zacchi, 2008; Stenberg, Tengborg, Galbe, & Zacchi, 1998; Tengborg et al., 1998; Wyman et al., 2009). In general softwoods have the lowest carbohydrate conversion rates due to their larger amount of lignin (Sjöström, 1993). Compared to the softwood reference, the total carbohydrate yield in this investigation is about 10% lower. The reduced yield could be explained by the fact that the cited softwood investigations were conducted with high quality wood, after debarking and further milling. In this study the raw material was more realistic, due to its low quality, the natural bark content, and the application of an industrial chipping process.

On basis of a total yield of 80% after steaming (see Table 4 Section 3.4) and a determined methane content of 60% in the produced biogas a methane yield of about 150 mL/g_{ODM} or 0.15 m^3/kg_{ODM} is obtained for the anaerobic fermentation of spruce forest residues. This is similar to results obtained for many organic fractions of municipal solid wastes (Nasir, Ghazi, & Omar, 2012). For hardwood higher yields can be expected due its lower lignin and higher carbohydrate proportion. The investigations indicate further on that by-products from the steam treatment e.g. furans are not inhibitive for the anaerobic digestion. This is a prerequisite for the utilisation of mixed wood residues for biogas production after the steam treatment. In the future the anaerobic co-fermentation of forest residues

with other substrates like organic municipal wastes should be further investigated. The utilisation of low quality mixed wood fractions e.g. hardwood and softwood from landscaping will be another future option in order to provide biogas.

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