Broeker, T., Hoffarth, M., Oppermann, L., Wolff, V., Neubauer, P. and Schneider, J.

Oxidation of Brewers Spent Grain for the Release of Fermentable Sugars by Direct Pretreatment in an Electrolysis Reactor

Brewer's spent grain (BSG) is a brewery side stream product available in large amounts that can provide so called drop-in compounds, sugars or ethanol. Technically challenging is the degradation of the lignin to release the carbohydrates (cellulose). Wet oxidation with active chlorine as a comparably gentle delignification method promises advantages compared with established harsh acidic or alkaline treatments. In case of brewer's spent grain (BSG), the consumption of oxidation agents by other organic non-lignin compounds may pose particularly difficulties. Therefore, a new method of oxidation pretreatment was introduced in which the substrate is placed directly into the electrolytic reactor to be permanently fed by oxidation agent. This technique was compared for the treatment of BSG and standard lignocellulosic substrates wheat straw (WS) and maize silage (MS) with an immersion in oxidation agent. The experimental setup consist of the two oxidative pretreaments (direct cell treatment and submerse treatment) followed by a standard enzymatic and fermentation process for the conversion of the released cellulose to sugar and alcohol. Oxidation of BSG and WS worked most efficiently at 4 °C and 20 °C at a pH value 7.0. The ratio of the active chlorine concentration in relation to biomass content in the treated mash was determined regarding the chlorine consumption rate and the efficiency of delignification. This ratio can therefore be optimised to obtain a rapid and efficient process. The active chlorine demand for BSG per dry matter substrate infeed was up to 810 mg/g while WS required only about 300 mg/g. A reduction of lignin up to 9.4 g/100 g d.m. was achieved for BSG, 10.7 g/100 g d.m for MS and even 17.7 g/100 g d.m. for WS. The direct treatment provided in 30 min a remarkable higher glucose yield for BSG and WS than the submerse treatment for 6 h. However, a 5 day submerse treatment reached and exceeded slightly the direct treatment results (0.20 g glucose per g dry matter biomass for BSG, 0.41 g/g d.m. for WS).

Descriptors: electrolysis, lignocellulose, pretreatment, wet oxidation, brewer's spent grain, fermentable sugars, wheat straw, bioeconomy

1 Introduction

1.1 Applications of brewer's spent grain (BSG) as a feedstock for bio-economy

Fossil based economy is sooner or later going to be substituted by bio based economy. Therefore, drop-in carbohydrates from renewables are desired by the chemical industry, often as a substrate for biotechnological applications.

Sidestreams from agriculture and food processing are potential sources for such a purpose. A major sidestream at industrial scale is brewer´s spent grain (BSG), which constitutes at least about 80–85 % of the organic by-products in breweries [1]. Approximately 3.4 million t of BSG are produced in European Breweries annually, 2 million t in Germany [2], [3].

Although the use as animal feed is an important application, alternatives lately became interesting within the last approximately 15–20 years. Besides increasing regulations for feed, growing demands of easily accessible sources for green economy feedstocks are reasons for this [2].

Hence, alternative applications have been subject of various research projects. *Musatto* et al. reflects potential such as energy production or human nutrition [4]. *Herfellner* investigated biogas application [5]. *McCarthys* review places emphasis on the potential as functional food ingredient [6]. *Steiner* et al. published a review on value-added polysaccharides derived from BSG for the food industry in reference to the EU health claims [2]. Mussato et al. investigated further the reuse of brewer's spent grain in chemical and biotechnological processes for the production of added-value compounds, such as xylitol, lactic acid and phenolic acids [7]. Energetic applications, combustion after drying were investigated by *Weger* et al. [8]. Also the use as biogas feedstock is widely described. Studies were carried out for example by *Ezeonu* and

Authors

Timo Broeker, Marc Hoffarth, Linda Oppermann, Verena Wolff, Jan Schneider, Institute of Food Technology ILT.NRW, University of Applied Sciences HS OWL, Lemgo, Germany; Peter Neubauer, Institute of Biotechnology, TU Berlin, Germany; corresponding author: timo.broeker@hs-owl.de

Abbreviations

$AC_{sp.\ biom.}$	Active chlorine consumption referred to biomass [mg/g d. m.]
$AC_{sp.\ glucose}$	Active chlorine consumption referred to glucose [g/g]
$AC_{sp.\ lignin}$	Active chlorine consumption referred to lignin[mg/g]
AFEX	Ammonia fibre explosion
BSG	Brewer´s spent grain
$c_{glucose}$	Glucose concentration [g /100 g]
CFU	Colony forming units [mL^{-1}]
d.m.	Dry matter
DCT	Direct cell treatment
DPD	N,N-Diethyl-p-phenylendiamin
ECAC	Electrolytic activated chlorine
HMF	Hydroxymethylfurfural
I	Electric current [A]
MS	Maize silage
p.p.	Percentage point
PP	Polypropylene
RA	Residual alkalinity [°dH]
R^2	coefficient of determination
TCV	Total viable count
WS	Wheat straw
Y	Yield [g /100 g]
$\chi_{biomass}$	Biomass (BSG, WS, MS) content in the oxidative solution [g d.m./100g]
$\chi_{lignin,0}$	Initial lignin concentration in the biomass [g/100 g d.m.]
$\chi_{lignin,\ f}$	Final lignin concentration in the biomass [g/100 g d.m.]

Okaka in 2006 [9] or Herfellner in 2007 [10]. *Aliyu* et al. reveal in their review the high potentials for biotechnological applications and the demands for intensive R & D activities in this field [3].

1.2 Pretreatment for delignification

BSG contain ash, fat, protein, cellulose, hemicellulose and lignin. The remaining carbohydrates are bounded in a structure of lignin. Many attempts have been made to obtain a suitable disintegration of the robust lignin structure in lignocellulose substrates to make the contained polysaccharides accessible. Since different lignocellulosic materials have different physico-chemical characteristics, it is necessary to adopt suitable pretreatments technologies based on the lignocellulosic biomass properties of each raw material [11].

Enzymatic hydrolysis of broken down lignocellulose works already quite successfully nowadays, as *Yang* and *Wyman* stated among others [12]. However, either costs or quality of the produced broth is preventing a feasible process from being provided in many cases. Not only the sugar yield, but also the fermentability of the solution is of importance. Inhibitors are either released during pretreatment as a result of the solubilised substances from the original biomass, such as acetic acid for example. Or inhibitors are formed in the pretreatment process, like furfural and 5-hydroxymethylfurfural (HMF), which are the result of degradation of carbohydrates [13], [14]. In further reactions, formic acid may be formed from furfural or HMF, which can also be converted to levulinic acid [15]. This often occurs at harsh conditions such as high temperature and pressure in pretreatment methods, like steam explosion, Ammonia Fibre Explosion (AFEX) or Organsolv [12], [16], [13].

Delignification experiments have been made with many substrates. Preatreatment of BSG has been investigated for either maximising biogas yields [5], [17] or the production of bioethanol, as reported by *White* et al. [18], who found inhibitory effects after dilute acid treatment of BSG.

1.3 Wet Oxidation of lignocellulose biomass

In contrast to, for example, steam explosion, ammonia fibre explosion (AFEX) or Organsolv, the wet oxidation method is described quite rarely in literature, although it has been proven to deliver good results for the disintegration of lignocellulose biomass and cellulose recovery up to 95–100 % [16], [19], [20]. Wet oxidation has also been studied on wheat straw [21] and on softwood [22], [23]. In general, low formation of inhibitors and efficient removal of lignin are achieved with wet oxidation pretreatment.

Delignification is mostly carried out at harsh conditions in established pretreatment methods, namely high temperature and pressure, often combined with high or low pH value. Amongst the known pretreatment methods the wet oxidation has basically the potential for an efficient lignocellulos degradation at comparably gentle conditions and hence low formation of inhibitors.

Oxidising the C = C bonds of lignin results in the destruction of the lignin without denaturising the carbohydrates to furfural and HMF [24], [13]. At the end of the treatment, pure cellulose remains. However, studies on oxidation pretreatments of lignocellulose have mostly been carried out at temperatures above 120–200 °C and overpressure of 2–20 bar, with the addition of sodium hydroxide, hydrogen peroxide or sodium carbonate at concentrations of 5–10 g L^{-1} [15], [14]. *Sugimoto* et al. reported good results using ozone as an oxidising agent [23]. *Sun* and *Cheng* stated advantages of ozonolysis pretreatment: (1) effectively remove of lignin; (2) no produce of toxic residues for the downstream processes; and (3) reactions are carried out at room temperature and pressure, but at the same time a requirement of large amounts of ozone, making the process expensive [25].

Energy costs and costs of oxygen and catalyst are considered one of the main disadvantages for those wet oxidation technologies [11].

Azzam worked at rather mild conditions using 2 % alkaline hydrogen peroxide at only 30 °C to solubilise about 50 % of lignin and most of the hemicellulose content of cane bagasse within 8 h [26]. The cellulose content was consequently increased from 42 % in the original cane bagasse to 75 % in the oxidized pulp.

Saccharification of the pulp residue with cellulases at 45 °C for 24 h yielded in a glucose content with 95 % efficiency [26].

Further, the oxidising agent chlorine is proven to react rapidly and extensively with lignin at ambient temperature [27]. *Richtzenhain*

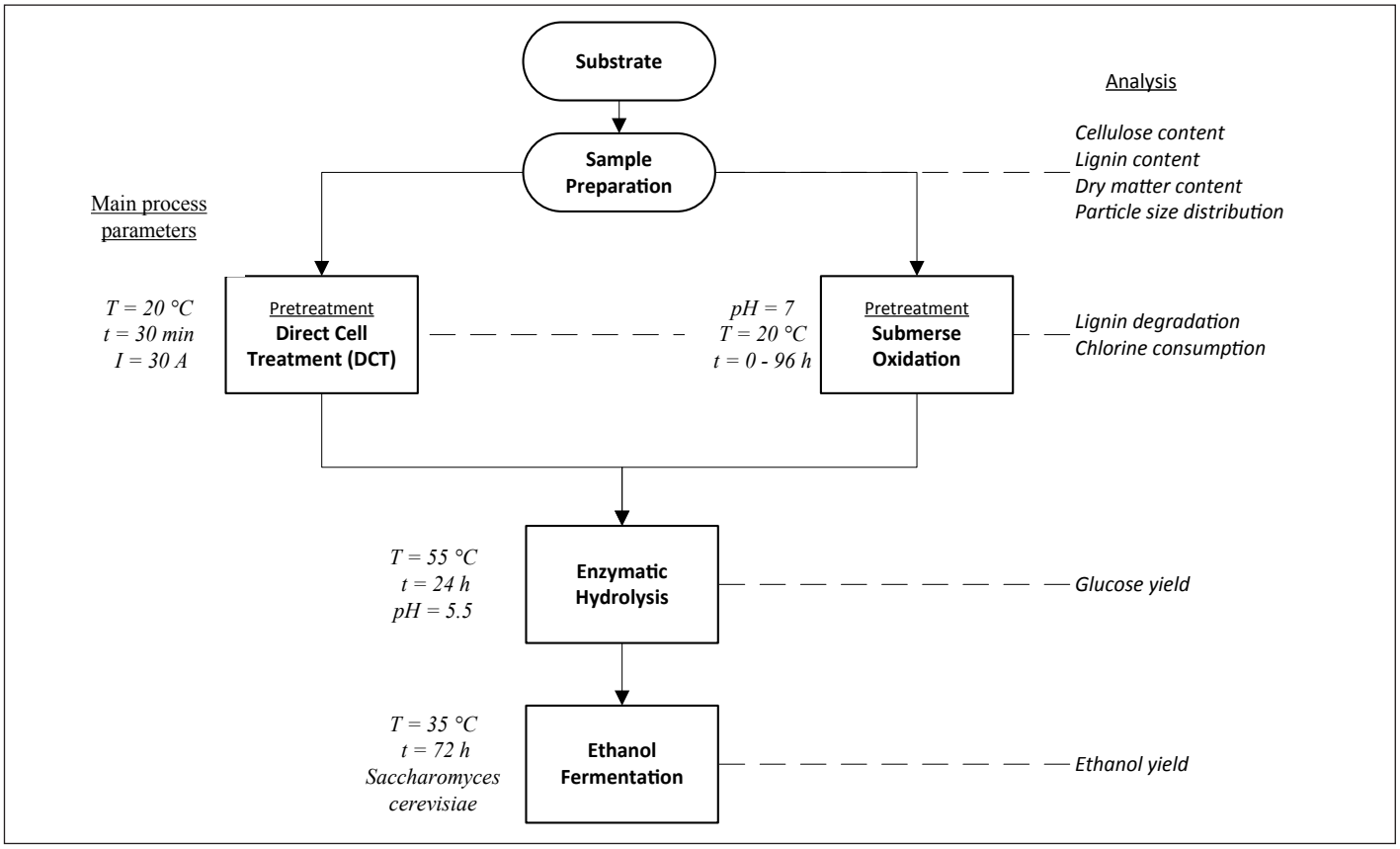

Fig. 1 Process in both variations and analysis; the setting of the parameters in the oxidation pretreatment was successively investigated as described in the text and finally set at 20 °C, pH 7, 24 h and 6000 ppm active chlorine for 1 g/100 g dry matter content

and *Alfredsson* were possibly the first ones to describe the disintegration of lignin by using hypochlorite in 1954 [28].

1.4 Electrolytic produced active chlorine (ECAC)

One main objective of this study was to investigate a wet oxidation process at ambient conditions. However, oxidation chemicals like pure H_2O_2, ClO_2, NaClO (Eau de Labarraque) or ozone are either unstable or expensive.

Consequently, it became interesting to study the possibility of using electrolytic produced active chlorine (ECAC) solutions, like they are used in water disinfection systems, in order to run a pretreatment on lignocellulosic biomass. The production of active chlorine from salt brine can be obtained by using titanium electrodes, which resulted in a solution with a concentration of about 6000 ppm of chlorine and derivatives such as NaClO, HClO, Cl_2O and Cl_2. This way, the chemicals are less expensive and produced on demand.

Xu et al. published results of the treatment of corn stover with an electrochemical system composed of a Ti/IrO_2–Pt plate as the anode and a Ti plate as the cathode [29]. They claimed that indirect oxidation by hypochlorous acid, which was generated in the bulk solution, was a significant contributor to lignocellulose oxidation, and that an increase in temperature and variation in pH during electrolysis enhanced the oxidation [29].

The brief survey of fundamentals indicates the starting point for the subsequent described investigation on the delignification of BSG with gentle and comparable more inexpensive wet oxidation procedures with and without direct treatment in an electrolysis reactor.

2 Materials and methods

2.1 General approach

Besides the usability of the process to release fermentable sugars, the feasibility of the wet oxidation and the developed direct cell method were of interest in this study.

The glucose yield, the amount of degraded lignin and the consumed concentration of oxidizing agents where investigated. Since there are no reported investigations on the oxidative pretreatment of BSG, forming of inhibitors from the high amount of contained protein or similar problems were taken into account. For this reason, other substrates were used for comparison. Since wheat straw is one of the major substrates to be investigated in order to deliver second generation cellulose from an agricultural sidestream, it was used as a model reference substrate. In order to provide another major biomass feedstock, maize silage was added to the assessments.

BSG and the two comparing substrates wheat straw and maize silage were initially prepared as described below. In a first series of trials, the parameter setting of the oxidation pretreatment in terms of temperature and pH value were adapted to the substrates. The-

Fig. 2 Picture of the opened electrolysis cell filled with BSG before treatment. The cell is closed with the upper electrode

refore the oxidation of the biomasses were conducted as submerse processes. As target figure, the ethanol yield was determined by employing a standard process consisting of an enzymatic hydrolysis and a *Saccharomyces cerevisiae* fermentation.

The kinetics of the chlorine consumption in dependence of the dry matter content of the biomass suspension were examined at the adjusted temperature and pH value in order to comparatively evaluate the process conditions and to sufficiently determine a ratio of chlorine and dry matter biomass content. The lignin content reduction was examined in the next step.

Finally, the submerse oxidation was compared with a direct treatment of BSG and other substrates in the electrolysis reactor DCT (direct cell treatment). Beside the glucose yield (after enzymatic treatment) and ethanol yield (after fermentation) the specific active chlorine consumption in relation the lignin degradation was observed.

2.2 Sample preparation

BSG were produced in a pilot scale plant. The grist load was 55 kg barley malt (25 kg Pilsner, 30 kg Vienna, Friability 90 %). The mashing with 180 L brewing liquor (RA 2.7 °dH) employed an infusion method (rests: 15 min at 52.0 °C, 30 min at 61.5 °C, 30 min at 71 °C, 1 min at 78 °C). An amount of 290 L water was used for sparging.

Wheat straw and maize silage were taken from a local farm and analysed for organic dry matter (d.m.) and fibre content. Spent grain and maize silage were then dried in a convection warming cabinet separately at 50 °C for 15 h. Since the straw did not undergo grinding before, it was milled (SM 2000, Retsch GmbH, Haan, Germany).

2.3 Wet oxidation reactor

Electrolytic activated chlorine (ECAC) was produced with a unique lab scale reactor (Figure 3), which was manufactured by aquagroup AG, Weiden, Germany. The electrodes of the electrochemical cell are made of titanium, the current is adjustable, as is the chlorine compounds concentration. The reactor produces up to 6 L h^{-1} of active chlorine from sodium chloride brine. The reaction chamber contains no membrane. It is built for the production of ECAC solution and optionally to be filled with a biomass sample.

In that way, the sample is perfused by the electrolysed solution and the reaction can permanently take place with the highest possible concentration of ECAC, which is an advantage compared to treating biomass submerse in a solution, where the concentration of active chlorine constantly decreases. This way, the delignification can be achieved faster with a higher space-time yield. The direct treatment of biomass within this electrochemical cell is subsequent abbreviated DCT (Direct Cell Treatment). Two different reaction techniques for pretreatment were used in this study: (1) Biomass substrate submerse treatment, which means it was suspended in ECAC for a certain time separated from the reactor; (2) a method for the DCT was designed to treat the samples directly in the reactor for 30 min at a current of 30 A and a voltage of 10 V. The

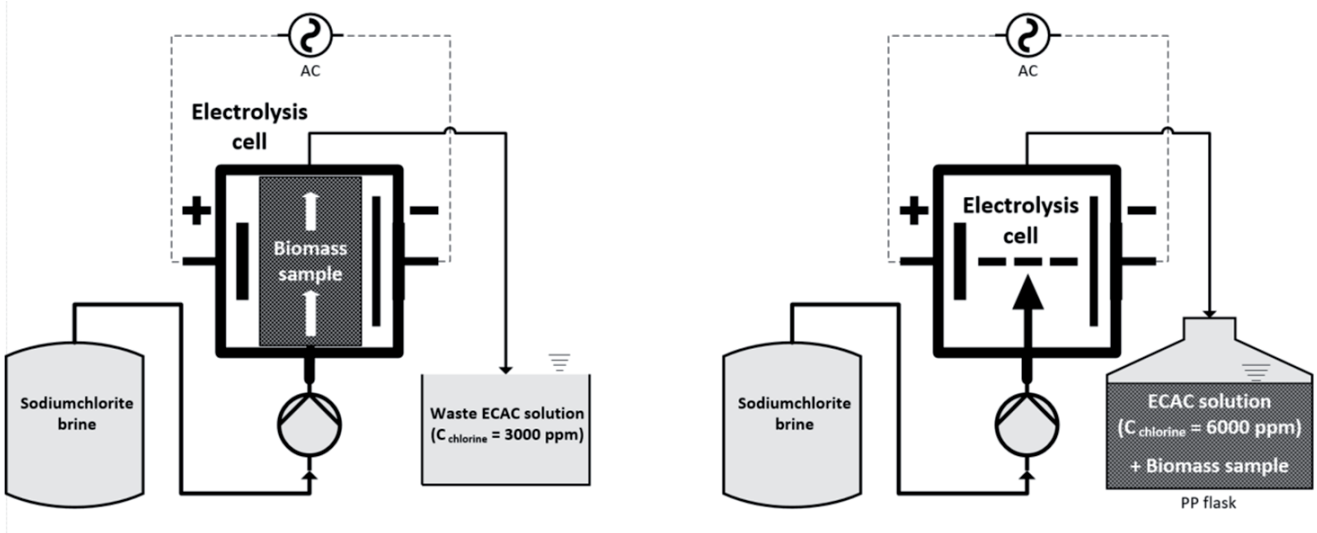

Fig. 3 Scheme of the electrolysis reactor and the two applied reaction techniques. Left – for direct cell treatment (DCT) of the biomass. It was filled in the cell between the electrodes. Sodium brine was pumped through the cell, in which it reacted and active chlorine was produced directly in the biomass. The waste solution from DCT was collected and the chorine content was analysed in the waste solution. Right – The brine was electrolysed without cell filling and the chlorine solution used in an external vessel for the pretreatment

current flows cross to the biomass bed. During the treatment, the polarity of the electrodes was reversed periodic to avoid burning of the biomass at the electrode surface, since chlorine is produced at the positive side. After this step, the samples were removed from the cell and due to the high solid content diluted with water for enzymatic hydrolysis.

For handling with ECAC, only plastic vessels and flasks (PP) were used in order to avoid any reaction, which can consume oxidising agent, like exchange of cations (for example from Mg, Zn or Al) out of the glass surface against the proton from oxonium ions.

2.4 Enzymatic hydrolysation and alcoholic fermentation

Cellulase Complex CELLIC CTec2 (Novozymes, Bagsvard, Denmark) was used for enzymatic hydrolysis of the cellulose. Maize silage was additionally treated with ENERZYME amylases (Erbslöh Geisenheim AG, Geisenheim, Germany) for the saccharification of the contained starch. The enzymatic saccharification was carried out in a lab shaker at 150 min^{-1} for 24 h at 40 °C and at pH 5.5 and dosage according to manufacturer's guideline and for CTec2 [30]. This enzymatic process was followed by a *Saccharomyces cerevisiae*, ENERFERM HT (Erbslöh Geisenheim AG) fermentation.

An amount of 0.5 g d.m. presuspended yeast was pitched into 200 g of the mash (2.5 % d.m. substrate) using 250 mL-Erlenmeyer flasks and adjusted to an pH value of 5-5.5 with NaOH or HCl (1 mol L^{-1}). The fermentation was conducted at a temperature of 35 °C for 72 h in an incubator with an orbital shaking (150 min^{-1}). This procedure has been elaborated as robust standard procedure for the evaluation of pretreatment methods, already applied for other kinds of pretreatment technologies, like acidic hydrolysis [31].

For the adaption of the sugar an ethanol conversion for the oxidative treatment it was necessary to verify whether the yeast is negatively impacted by chlorine residues. Therefore the yeast (*Saccharomyces cerevisiae*, ENERFERM HT, $5.92 \cdot 10^{13}$ CFU mL^{-1}) was exposed to different concentrations of the oxidation agent. As expected, in presence of already low chlorine concentrations (lowest tested concentration 100 ppm) no viable cells could be found. In addition, the active chlorine reduction in presence of glucose. If 5 % glucose was added to the concentrated ECAC solution for 24 h at 20 °C the concentration of free chlorine found was < 30 ppm. After alcoholic fermentation, a decreased ethanol yield of 17 % was obtained. In consequence of these findings, it was necessary to separate the chlorine solution prior to enzymatic treatment and fermentation for low biomass concentrations like 1.0 % and lower. The investigation of wet oxidation parameter settings as shown in table 2 was carried out at a biomass concentration of 7.5 % d.m. without a washing and drying step with direct fermentation of the ECAC mash.

2.5 Chemical analysis

Active chlorine

Two methods for the quantification of free chlorine were necessary, one for the clear solutions and a second for solid matter containing samples. The clear chlorine solution from the reactor for the submerse incubation was determined with a photometric assay by measuring the extinction of DPD (N,N-Diethyl-p-phenylendiamin) with a PCCOMPACT (Aqualytics, Germany) photometric system. The sample was diluted 1:2000 and 10 mL taken to treat the DPD and measure the extinction. Similar results were obtained using a photometric LCK Test (Hach Lange, Düsseldorf, Germany). The colouring agent in this method is N-N-Diethyl-1,4-phenylendiammoniumsulfate. The Photometer used was a CADAS 100 (Dr. Bruno Lange GmbH, Germany).

Due to the turbidity, biomass containing samples ("mash") cause problems for photometric quantification. For samples with a high solid content, an assay for iodometric titration was adapted and optimized for the samples.

During the titration, a redox reaction takes place. By adding iodide, the active chlorine in the solution is reduced to chloride while the iodide is oxidised to iodine. The reaction balance shifts over completely to the side of the products at acidic pH value. The iodine produced in this way can be reduced with sodium thiosulfate ($Na_2S_2O_3$) to Iodide. The created assay was carried out as follows:

A 250 mL-Erlenmeyer flask was filled with 100 mL of the diluted sample (1:5) together with 20 ml hydrochloric acid (c = 2 mol L^{-1}) and 6–8 mL potassium iodide solution (10 % w/v). It was titrated with sodium thiosulfate (c = 0.05 mol L^{-1}) to bright yellow and then coloured dark blue with 1 ml of starch solution (c = 1 %). The solution was titrated with sodium thiosulfate until the colour paled. The volume of the consumed sodium thiosulfate $Na_2S_2O_3$ (x) was used to calculate the concentration of active chlorine.

Statistical quality of the assays: within nine determinations, the (i) Aqualytics assay delivered results with standard deviation of 0.1 %, (ii) the LCK Test delivered 2.3 % and (iii) the titration 1.0 %. The statistics of the methods were examined separately, ahead of the trials. This was done regarding the proceeding reaction of the oxidation during sampling. Analysis had to be carried out in an equivalent period of time. Analysing multiple samples would have had a negative influence on the results.

Fibre analysis

The Klason Lignin content was detected gravimetrically as non-digestible fibre after acid treatment and washing steps, as described, for example, by *Lin* and *Dence* [32]. The cellulose content determination was carried out by LUFA Nord-West, Oldenburg, Germany according to the VDFLUFA [33].

Particle size analysis

The particle size was analysed by sieve analysis, n = 3, using Retsch sample test sieves and shaker.

Glucose and ethanol concentration

The sugar content was determined with Flexar HPLC System, RI Detector (Perkin Elmer, USA), on NUCLEODUR 100-5 NH2-RP, 5 µm particle size (Macherey Nagel, Düren, Germany), eluent

in column acetonitrile – water, or by using enzyme assays on a Gallery Automated Photometric Analyser (Thermo Scientific, USA). Ethanol was determined by measuring the density (DMA, Anton Paar, Graz, Austria) after distillation.

2.6 Characteristic figures and statistical analysis

Glucose yield

The glucose yield was estimated as the coefficient of the measured glucose concentration $c_{glucose}$ versus the content of dry matter biomass $\chi_{biomass.}$

$$Y_{glucose/biomass} = \frac{c\ glucose\ in\ g/100\ g}{\chi biomass\ in\ g\ d.m./100\ g} \qquad Eq.\ 1$$

Lignin degradation

The proportionate lignin reduction $\Delta\chi_{lignin}$ in $\frac{g}{100}g\ (d.m.)$ is an interesting accompanying information in order to see how much of the lignin structure has to be dissolved for the obtained glucose yield. The figure is built as difference of initial lignin concentration $\Delta\chi_{lignin,\ o}$ and after the oxidation process $\Delta\chi_{lignin,\ f}$

$$\Delta\chi_{lignin} = \chi_{ligning,0} - \chi_{ligning,f} \qquad Eq.\ 2$$

Specific active chlorine consumption

For the evaluation of the BSG in comparison to the other substrates and the comparison of the two investigated reaction techniques (DCT and submers), the specific active chlorine consumption is of great interest. Therefore, the consumption of active chlorine as concentration difference before and after oxidation treatment was referred to the mass of glucose yielded $AC_{sp.\ glucose}$, the mass of lignin reduced $AC_{sp.\ lignin}$ and the biomass substrate mass (d.m.) $AC_{sp.\ biomass}$. The active chlorine consumption in case of the latter figure was extracted from the kinetic trials as subsequently described.

Oxidation rate

The degradation of lignin and other oxidisable compounds can indirectly be observed by the active chlorine consumption. The decline of chlorine follows sufficiently an exponential decay function starting from the initial concentration c_0 towards a minimum $c_{\infty.}$

$$c(t) = c_\infty + c^* \cdot e^{-k \cdot t} \qquad Eq.\ 3$$

The coefficient $c^* = c_0 - c_\infty$ represents the consumption of chlorine. In order to find appropriate processing conditions and to verify the delignification, the specific consumption was observed as the ratio of c* versus the dry matter content of the biomass suspension $x_{d.m.}$:

$$AC_{sp.\ biomass} = \frac{c^*}{x_{d.m.}} \qquad Eq.\ 4$$

A second key figure is the rate of chlorine consumption as measure represented be the constant *k*. The chlorine consumption rate *k* indicates further process parameters, particularly the temperature and mass transfer influencing sizes as the particle sizes and the hydrodynamic conditions. Thus, this figure gives an information about the different investigated reactions methods.

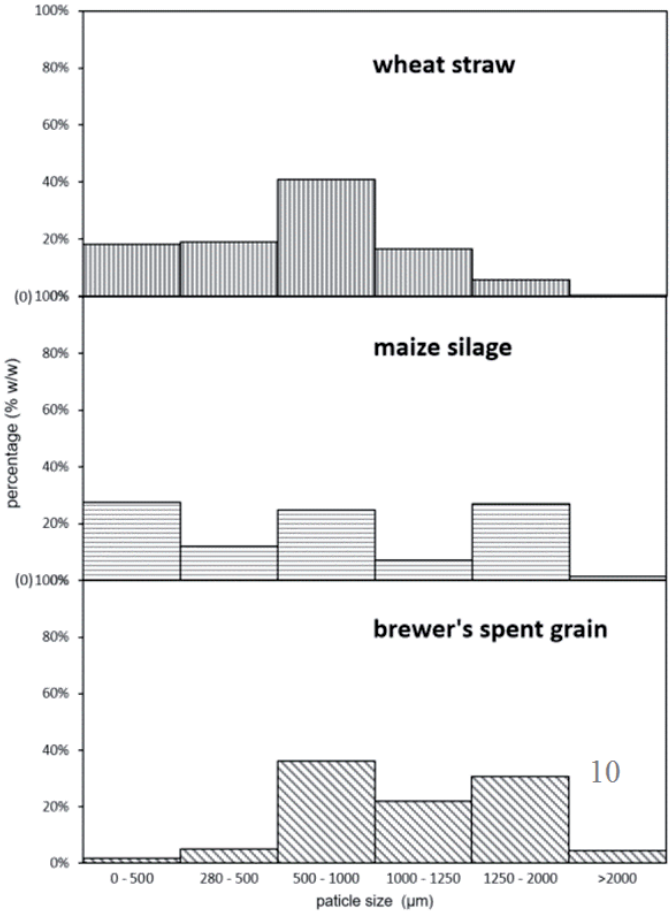

Fig. 4 Particle size distribution of the samples

Statistical analyses including regression analysis were carried out using OriginPro 2017G (OriginLab Cooperation, Northampton, USA) to determine coefficients of determination, mean values and standard deviations.

3 Results and Discussion

3.1 Characterization of substrates

The oxidation and kinetics of the lignin degradation might be influenced by grain sizes and surface proportions.

Table 1 Cellulose, Lignin and Dry matter content (after sample preparation) of substrates (BSG – brewer´s spent grain, WS – wheat straw, MS – maize silage)

	Cellulose (g/100 g d.m.)	Lignin (g/100 g d.m.)	Dry matter content (g/100 g)
BSG	21,8	22.5	90.2
WS	38,6	24.3	91.7
MS	19.2	19.9	92.3

Table 2 **Influence of temperature and pH-value settings in submerse wet oxidation pretreatment (initial chlorine concentration 6000 ppm) on the ethanol concentration (as mean value χ and range as R = χ_{max} – χ_{min}) after a standard enzymatic hydrolysis and alcoholic fermentation, carried out on wheat straw as model substrate. The content of dry matter was 7.5 %. Fermentation was done directly in the mash without further separation**

ph value	Tempera-ture	Ethanol concentra-tion	Range	Sample size
(–)	(°C)	(% w/w)	(% w/w)	(–)
5	4	0.21	0.04	3
5	20	0.22	0.02	3
5	50	0.25	0.08	4
7	4	0.28	0.07	5
7	20	0.27	0.08	7
7	50	0.24	0.02	3
9	4	0.20	0.09	5
9	20	0.22	0.01	3
9	50	0.23	0.05	5

The following steps of standard enzymatic and fermentation process might be affected as well. Figure 1 shows the results of the particle size distribution of the samples.

The results of the substrate analysis in table 1 were used for the determination of characteristic numbers as the specific chlorine consumption per biomass or lignin and the specific glucose yield.

The dry matter content is given for the dry samples after preparation.

3.2 Wet oxidation parameter settings

Before comparing different reaction techniques and substrates, the process parameters pH value and temperature were investigated with 39 settings. The target figure was the ethanol concentration after standardised enzymatic hydrolysis and fermentation.

The most successful settings for the substrates were 4 °C at a pH value of 7.0. However, the settings for further trials were adjusted at pH value 7 and room temperature for practical reasons. Considering the deviations, the ethanol yield was almost comparable.

3.3 Investigation on the concentration ratio of biomass and active chlorine solution

The ratio of biomass substrate and ECAC solution influences the rheological properties of the suspension and thus the processability. As part of this issue, the ratio must be balanced in that way, that neither a lack nor an inappropriate surplus of active chlorine is provided. High substrate concentrations in the ECAC solutions

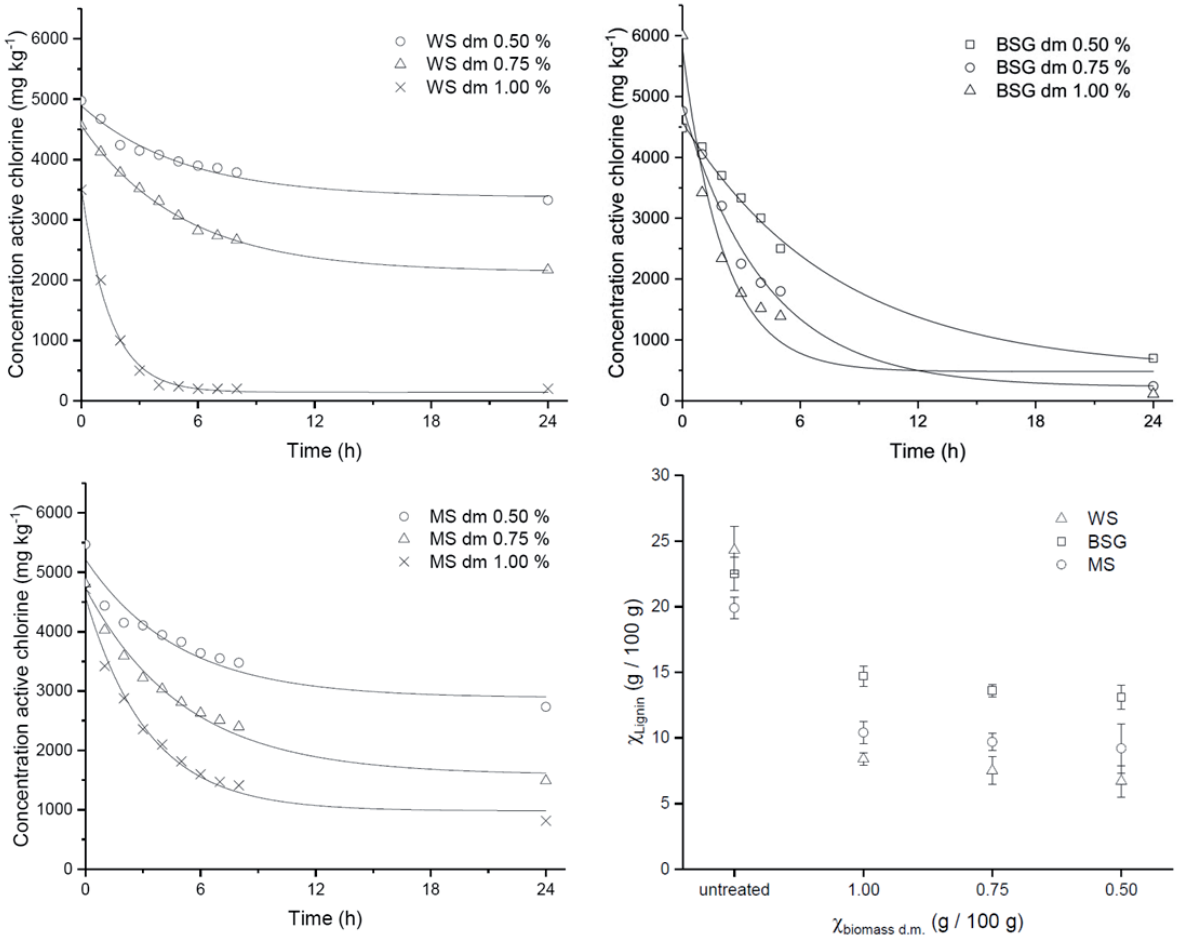

Fig.5 **Kinetics of active chlorine consumptions in different concentrations of wheat straw (WS – upper left), maize silage (MS – lower left) and brewer's spent grain (BSG – upper right) by submerse ECAC-treatment at pH value 7 and 20 °C. The correlated Lignin content (lower right) of the untreated sample and after 24 h indicates the effect of the oxidisation**

Table 3 **Characteristic figures (as described in Materials and Methods) corresponding to the trials presented in Figure 5. The figures are used to evaluate the kinetics and process conditions of the active chlorine oxidation process. The consumption of chlorine is listed for each substrate and concentration of dry matter**

	$\chi_{d.m.}$ (g/100 g)	c* (mg/kg)	k (h^{-1})	R^2_k (-)	$AC_{sp.\ biom.}$ (mg/g)	$\Delta\chi_{Lignin}$ (g/100 g)	$AC_{sp.lignin}$ (mg/g)
WS	0.50	1517	0.190	0.956	303	17.7	8.6
	0.75	2427	0.194	0.997	324	16.8	14.4
	1.00	3404	0.680	0.995	340	16.0	21.3
MS	0.50	2330	0.207	0.944	466	10.7	21.8
	0.75	3145	0.195	0.981	419	10.3	30.7
	1.00	3600	0.306	0.989	360	9.5	37.8
BSG	0.50	4080	0.128	0.993	816	9.4	43.4
	0.75	4621	0.237	0.985	616	8.9	51.9
	1.00	5311	0.478	0.959	531	7.8	67.8

of 2.0 % and 2.5 % could still be handled in terms of their fluid-mechanical properties. However, the chlorine decay at these high substrate concentrations was thus rapid that the active chlorine was instantly consumed and a course of the consumption over the time could not be measured. Therefrom, data shown in Figure 5 represent only substrate concentrations up to 1.0 %.

While dry matter concentrations of 0.5, %, 0.75 % and 1.0 % biomass were exposed to ECAC solution, the kinetics of the chlorine consumption were observed. At 1.0 % d.m. content in case of straw and BSG, the results showed that the demand for oxidation agent was higher than provided to the biomass. In all cases of the 0.5 % concentration, the decrease of the chlorine concentration approached nil. This allowed to estimate the specific consumption rate of chlorine per g biomass, as pointed out in table 3.

The coefficient k indicates the rate of the oxidation reaction. The coefficients of determination R^2 for the linearized data confirm the kinetic satisfactorily. As expected, the rate can be enhanced by higher substrate concentrations. However, a high rate indicates the total consumption of chlorine while unoxidated biomass remains.

The chlorine demand of BSG is the highest, while both BSG and maize silage consume more than wheat straw. This might be due to the composition of BSG and also maize silage. In contrast to straw they contain oxidisable compounds, especially proteins, and are very likely to consume chlorine without adding value to the determined objective criteria. However, MS and BSG indicate a lower chlorine demand at higher substrate concentrations. This correlates with the amount of reduced lignin $\Delta\chi_{lignin}$, which is higher for wheat straw.

In all cases, that the approached chlorine concentration minimum reaches zero, it can be assumed that there is still oxidisable substrate and the chlorine amount was too low. A suitable ratio of biomass and active chlorine must therefore provide the steepest possible chlorine decay just not reaching a minimum of zero.

Wheat straw shows the lowest consumption of active chlorine and the highest lignin disintegration. Since wheat straw consists of mostly lignocellulose and in contrast to the other biomasses a low amount of protein, the chlorine is mostly consumed by the delignification and not from other oxidisable substances.

Although the common assay for Klason - lignin analysis is frequently used in biomass experiments and sufficiently reproducible, absolute values might vary in the described experimental setup. Since the substrates were treated submerse in chlorine solution, before dried and analysed, soluble components like protein are very likely washed out, so that the relative lignin content in relation to the untreated reference sample is higher. Nevertheless, the high reproducibility of the assay allows to observe the lignin value as an indicator for efficiency of the degradation.

3.4 Comparison of submerse oxidation and direct electrochemical cell treatment (DCT)

The comparison of the reaction techniques (DCT and submerse) were conducted with BSG and for comparison with WS. The residence time in direct cell treatment (DCT) was 30 minutes at 30 A, the submerse treatments lasted 6 h and as long term comparison 120 h. As result from at least 3–6 performances, the mean characteristic numbers are shown in Table 4. The samples were hydrolysed by enzymes in the next step. The Glucose yield was observed in relation to the consumed chlorine.

Exposing the biomasses to the ECAC solution for 120 h lead to glucose yields of more than 91 %, when referred to the approximate cellulose content. The long reaction time results consequently in the lowest consumption for active chlorine.

The DCT method appears to have advantages in comparison to the submerse method. This way, maximum active chlorine concentration (approx. 6000 ppm) was provide to the biomass over the entire reaction time (30 min.). This resulted in the highest yield related to the treatment time. The DCT process resulted for both WS and BSG in a glucose yield between 73 % (WS) and 79 % (BSG), which was significantly higher than with the 6 h submerse treatment. However, the demand of active chlorine in relation to released glucose appears in both cases to be about twice as high as in case of the 120 h submerse treatment.

Table 4 **Characteristic figures as result of submerse and direct cell oxidation of wheat straw (WS) and brewer´s spent grain (BSG), shown are the arithmetic mean from at least 3–6 performances; initial ECAC concentration 6000 ppm, pH value 7.0, 20 °C**

		$Y^{glucose}/_{biomass}$ (g/g d.m.)	$AC_{sp.\ biomass}$ (g/g)	$AC_{sp.\ glucose}$ (g/g)
BSG	DCT	0.17	1.37	8.08
	6 h	0.09	0.46	5.00
	120 h	0.20	0.82	4.06
WS	DCT	0.28	1.39	4.92
	6 h	0.16	0.34	2.10
	120 h	0.37	0.68	1.83

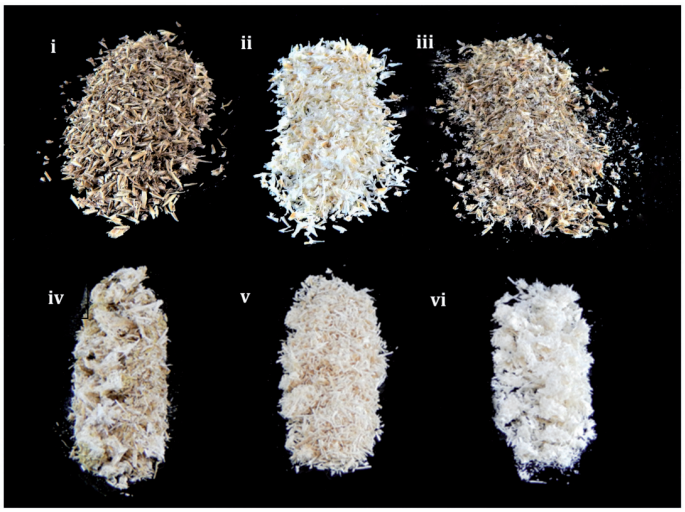

Fig. 6 Bleaching effect and the visible grade of released cellulose of active ECA chlorine on BSG and wheat straw. i: untreated spent grain; ii: BSG 120 h; iii: BSG DCT 0,5 h, iv: WS DCT 30 min; v: WS 6 h; vi: WS 120 h

Due to the flowrate of the brine through the cell, the impact of chlorine is higher towards the outlet. Along with the periodic reverse of the polarity of the electrodes, the flow direction should be changed to avoid a concentration gradient and inhomogeneous results.

Due to the high cellulose content, wheat straw shows a better applicability for the production of sugars then BSG. Besides the higher glucose yields, the consumption of active chlorine for the disintegration of lignin and the liberation of glucose is only half of BSG.

Soluble hemicellulose might be washed out in the DCT method, which could be a disadvantage. However, a suitable dosage of active chlorine, that allows the complete consumption of oxidation potential, offers the possibility for recovery of xylose from the ECAC waste fraction. As with many other delignification methods, the lignin cannot be used as a solid fuel, which for some biorefinery concepts considerably reduces the income from by-products in large-scale production, as claimed, for example, by *Galbe* et al. [34].

The impact on the delignification through the three diffenrent oxidativ treatments results in visible bleeching of the biomass samples (Figure 6). The samples with the most intense visible release of cellulose deliver the glucose yields of over 90 %.

In contrast to the good results concerning the delignification and saccharification, the fermentation of the glucose to ethanol delivered poor conversion efficiency. While in former studies with an acidic hydrolysis pretreatment and similar saccharification and fermentation, yields of 11 % EtOH (d.m.) for wheat straw (80 % of maximum possible yield) and 10 % for spent grain have been reached [31], the fermentation of oxidised samples in this study resulted in maximum of 3.7 % EtOH yield. A reasons might be the disinfecting impact of remaining chlorine in the mash with low dry matter content on the side. The poor delignification in the 7.5 % d.m. higher solid mash is a possible reason on the other side, so that further investigations on alcoholic fermentation have to be carried out.

To give an orientation about the comparison with reported pretreatment, Table 5 shows results as reported in the review by *Maurya* et al. in 2015.

4 Conclusion/summery

Complete delignification at low concentrations of oxidative chemicals is possible. The feasibility of the delignification of brewer's spent grain and other lignocellulosic material in a direct cell treatment reactor as well as in a submerse treatment could be shown. Glucose yield for wheat straw was ~90 % of the theoretical maximum. A high sugar yield indicates that the enzymatic accessibility of lignocellulose is enforced by process. Therefore, it could be shown that ECAC fulfils the demands for a suitable pretreatment, ("yields higher than 90% in less than five and preferably less than 3 days with enzyme loading lower than 10 FPU/g cellulose") claimed, for example, by Yang and Wyman for wheat straw [12].

Table 5 Results in comparison with other pretreatments as reported by Maurya et al. [35]

Pretreatment	Biomass	Lignin removal	total reducing sugar	Source
DCT	WS BSG	-	73% 79%	-
Submers ECAC	WS BSG	72% 42%	91%	-
Ozonolysis pretreatment	Japanese cedar	-	69%	Miura et.al. (2012)
glycerol-based autocatalytic organosolv	wheat straw	65%	-	Sun and Chen 2008
microwave-based alkali pretreatment	switchgrass	-	70–90 %	Hu and Wen 2008
microwave-assisted ammonium hydroxide	Miscanthus sinensis	74%	41%	Boonma-numsin et al. (2012)
aqueous ammonia and dilute H_2SO_4	rice straw	-	90.8 %	Kim et al. (2011)
steam pretreatment with diluted H_2SO_4	corn stover	-	78%	Bondesson et al. (2013)
diluted H_2SO_4	Eulaliopsis bi-nata	-	21.02 %	Tang et al. 2013

The feasibility of the delignification of BSG and other lignocellulosic material in a direct cell treatment reactor as well as in a submerse treatment could be shown. The process condition during oxidation at 20 °C and pH 7.0 are suitable for BSG, WS and MS. The active chlorine concentration and the biomass content in the treated mash must be individually adjusted in order to obtain both, a rapid process and a high extent of lignin degradation. The direct cell treatment accelerates the glucose yielding process compared with the immersion in oxidation agent. However, the consumption of oxidation agent is higher. The process works well for BSG to provide glucose, but straw performs remarkably better likely due to less oxidation agent consuming non-lignin compounds as protein. Ethanol fermentation did not perform comparably and as expected, possibly because of residues of the

oxidation agents which may affect the yeast activity. Here, further effort seem to be required to investigate if and how the transition from oxidation to the enzymatic and fermentation process can be improved. Also, a possible chlorate formation in the off stream should be taken into account.

Acknowledgement

The project was funded within the ZIM – Program by the Federal Ministry for Economy and Energy BMWi (KF2402603BN3).

The cooperation project was carried out together with aquagroup AG, Weiden, Germany, who built the process equipment for the ECAC – application.

5 Conflict of interest

The authors declare no financial or commercial conflict of interest.

6 References

1. Herfellner, T.; Bochmann, G. and Meyer-Pittroff, R.: Wirtschaftlich sinnvolle Verfahren?: Die Verwertung von Biertrebern – derzeitiger Stand und neue Ansätze zur energetischen Nutzung, Brauindustrie (2006), no. 8, pp. 42-45.
2. Steiner, J.; Procopio, S. and Becker, T.: Brewer's spent grain: Source of value-added polysaccharides for the food industry in reference to the health claims, European Food Research and Technology, **241** (2015), no. 3, pp. 303-315.
3. Aliyu, S. and Bala, M.: Brewer´s spent grain: A review of its potentials and applications, African Journal of Biotechnology **10** (2011), no. 3, pp. 324-331.
4. Mussatto, S. I.; Dragone, G. and Roberto, I. C.: Brewers' spent grain: Generation, characteristics and potential applications, Journal of Cereal Science, **43** (2006), no. 1, pp. 1-14.
5. Herfellner, T.: Anaerobe Hydrolyse und Methanisierung fester, flüssiger und pastöser organischer Produktionsrückstände aus Brauereien, dissertation, Technische Universität München, 2010.
6. McCarthy, A. L.; O'Callaghan, Y. C.; Piggott, C. O.; FitzGerald, R. J. and O'Brien, N. M.: Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review, The Proceedings of the Nutrition Society, **72** (2013), no. 1, pp. 117-125.
7. Mussatto, S. I.; Dragone, G.; Teixeira, J. A. and Roberto, I. C.: Total reuse of brewer's spent grain in chemical and biotechnological processes for the production of added – value compounds, International Conference and Exhibition on Bioenergy, Guimarães, 2008.
8. Weger, A.; Binder, S.; Franke, M.; Hornung, A.; Ruß, W. and Mayer, W.: Solid Biofuel Production by Mechanical Pre-Treatment of Brewers' Spent Grain: IconBM, International Conference on BioMass : 4-7 May 2014, Florence, Italy, Chemical Engineering Transactions, volume 37 (2014).
9. Ezeonu, F. C. and Okaka, A.: Process kinetics and digestion efficiency of anaerobic batch fermentation of brewer's spent grains (BSG), Process Biochemistry, **31** (1996), no. 1, pp. 7-12.
10. Herfellner, T.: Anaerobe Hydrolyse und Methanisierung fester, flüssiger und pastöser organischer Produktionsrückstände aus Brauereien, dissertation, Technische Universität München, München, 2011.
11. Alvira, P.; Tomás-Pejó, E.; Ballesteros, M. and Negro, M. J.: Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, Bioresource Technology, **101** (2010), no. 13, pp. 4851-4861.
12. Yang, B. and Wyman, C. E.: Pretreatment: The key to unlocking low-cost cellulosic ethanol, Biofuels, Bioproducts and Biorefining, **2** (2008), no. 1, pp. 26-40.
13. Taherzadeh, M. J. and Karimi, K.: Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review, International Journal of Molecular Sciences, **9** (2008), no. 9, pp. 1621-1651.
14. van der Pol, Edwin C.; Bakker, R. R.; Baets, P. and Eggink, G.: By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio)chemicals and fuels, Applied Microbiology and Biotechnology, **98** (2014), no. 23, pp. 9579-9593.
15. Olsson, L. and Ahring, B. K.: Biofuels, Springer, Berlin, New York, 2007.
16. Carcalheiro, F.; Duarte, L. C. and Girio, F. M.: Carvalheiro, F., Duarte, L.C., Gírio, F.M., 2008. Hemicellulose biorefineries: a review on biomass pretreatments, J. Sci. Ind. Res., **67** (2008), pp. 849-864.
17. Macheiner, D.; Adamitsch, B. F.; Karner, F. and Hampel, W. A.: Pretreatment and Hydrolysis of Brewer's Spent Grains, Engineering in Life Sciences, **3** (2003), no. 10, pp. 401-405.
18. White, J. S.; Yohannan, B. K. and Walker, G. M.: Bioconversion of brewer's spent grains to bioethanol, FEMS yeast research, **8** (2008), no. 7, pp. 1175-1184.
19. Schmidt, A. S. and Thomsen, A. B.: Optimization of wet oxidation pretreatment of wheat straw, Bioresource Technology, **64** (1998), no. 2, pp. 139-151.
20. Talebnia, F.; Karakashev, D. and Angelidaki, I.: Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation, Bioresource Technology, **101** (2010), no. 13, pp. 4744-4753.
21. Klinke, H. B.; Olsson, L.; Thomsen, A. B. and Ahring, B. K.: Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of *Saccharomyces cerevisiae*: Wet oxidation and fermentation by yeast, Biotechnology and Bioengineering, **81** (2003), no. 6, pp. 738-747.
22. Palonen, H.; Thomsen, A. B.; Tenkanen, M.; Schmidt, A. S. and Viikari, L.: Evaluation of Wet Oxidation Pretreatment for Enzymatic Hydrolysis of Softwood, Applied Biochemistry and Biotechnology, **117** (2004), no. 1, pp. 1-18.
23. Sugimoto, T.; Magara, K.; Hosoya, S.; Oosawa, S.; Shimoda, T. and Nishibori, K.: Ozone pretreatment of lignocellulosic materials for ethanol production: Improvement of enzymatic susceptibility of softwood, Holzforschung, **63** (2009), no. 5.
24. Bjerre, A. B.; Olesen, A. B.; Fernqvist, T.; Plöger, A. and Schmidt, A. S.: Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose, Biotechnology and Bioengineering, **49** (1996), no. 5, pp. 568-577.
25. Sun, Y. and Cheng, J.: Hydrolysis of lignocellulosic materials for ethanol production: A review, Bioresource Technology, **83** (2002), no. 1, pp. 1-11.
26. Azzam, A. M.: Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation, Journal of Environmental Science and Health, Part B, **24** (1989), no. 4, pp. 421-433.
27. Dence, C. W. (Ed.): The determination of Lignin, Springer-Verlag, Berlin, New York, 1992.
28. Richtzenhain, H.; Alfredsson, B.; Lang, W. and Berndt, W.: Über den Abbau von Lignin und Ligninmodellsubstanzen mit Hypochlorit. II.

Mitteilung, Acta Chemica Scandinavica, **8** (1954), pp. 1519-1529.
29. Xu, B.; Zhang, B.; Li, M.; Huang, W.; Chen, N.; Feng, C. et al.: Production of reducing sugars from corn stover by electrolysis, Journal of Applied Electrochemistry, **44** (2014), no. 7, pp. 797-806.
30. Lan, T. Q.; Lou, H. and Zhu, J. Y.: Enzymatic Saccharification of Lignocelluloses Should be Conducted at Elevated pH 5.2–6.2, BioEnergy Research, **6** (2013), no. 2, pp. 476-485.
31. Broeker, T.; Steffens, M.; Blöhse, D.; Schulze, C. and Schneider, J.: Delignification of brewery spent grains for bioethanol production: Poster presented at 33rd EBC Congress, European Brewery Convention, Glasgow, May 22, 2011.
32. Lin, S. Y. and Dence, C. W.: Methods in Lignin Chemistry, Springer, Berlin, Heidelberg, 1992.
33. Naumann, C. and Bassler, R.: Die chemische Untersuchung von Futtermitteln, vol. 3, VDLUFA-Verl., Darmstadt, 2012.
34. Galbe, M.; Sassner, P. and Wingren, A., Zacchi, G.: Process Engineering Economics of Bioethanol Production, Springer, Berlin, New York, 2007.
35. Maurya, D. P.; Singla, A. and Negi, S.: An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol, 3 Biotech, **5** (2015), no. 5, pp. 597-609.

Received 2 March 2017, accepted 14 April 2017