

Investigation of Pre-Treatment Parameters of Rye and Triticale for Bioethanol Production

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Abstract—This paper presents the new results of energy plant – rye and triticale at yellow ripeness and ripe, pre-treatment in high pressure steam reactor and monosaccharide extraction. There were investigated the influence of steam pressure (20 to 22 bar), retention duration (180 to 240 s) and catalytic sulphuric acid concentration strength (0 to 0.5 %) on the pre-treatment process, contents of monosaccharides (glucose, arabinose, xylose, mannose) and undesirable by-compounds (furfural and HMF) in the reactor. The study has determined that the largest amount of monosaccharides (37.2 % of glucose, 2.7 % of arabinose, 8.4 % of xylose, and 1.3 % of mannose) was received in the rye at ripe, the samples of which were mixed with 0.5 % concentration of catalytic sulphuric acid, and hydrolysed in the reactor, where the pressure was 20 bar, whereas the reaction time – 240 s.

Keywords—Bioethanol, Pre-treatment, Rye, Triticale.

I. INTRODUCTION

WITH the increase of reduction of the solid fuel reserves and atmosphere pollution, the alternative energy sources acquire increasing the actual value. The ethanol is one of these sources. Bio-ethanol can be used for burning, as a fuel and in chemical industry, as a raw material. More wide use of bio-ethanol is impeded by the great cost of the hydrolysis in production process. The production of bio-ethanol is especially developed in USA, Brazil and other countries [1].

The main raw materials for bio-ethanol production are sugar and biomass having big amount of sugar (sugar beet, potatoes, etc.), and big amounts of these raw materials could be used for bio-ethanol production. Big problem of these raw materials usage for bio-ethanol production – the costs of these materials comprise 40-60 % of all production costs [1]. The alternative material for bio-ethanol production can be used the biomass, which have big amount of cellulose (wood, straw, etc.). These raw materials have comparatively small price are can be used how a source of bio-ethanol production from biomass [2].

The biomass production process from bioethanol is classified into the following production stages: raw material preparation, initial hydrolysis, hydrolysis, fermentation, distillation, and dehydration.

The present paper presents a rye and triticale biomass preparation and examines only the initial biomass hydrolysis, which takes place, when the biomass is affected by a high-pressure steam [3].

When the cellulose of biomass is hydrolyzed, big attention should be paid to disarrangement of fibrous biomass structure. It could be effectively done by usage of the high pressure steam. Biomass disruption by usage of high pressure steam was first tested in 1925, and in the seventh decade of the XX century this method was used to prepare biomass for hydrolysis. Ditrach et al. [4] fulfilled the tests with chopped wood, and the best results were achieved when the reactor temperature was from 185 to 190°C (the duration of hydrolysis process was 8 min.). Marchesault et al. [5] managed to separate cellulose, hemi-cellulose and wooden wool fractions from aspen wood by usage of high pressure steam.

When biomass is mixed with mineral acids (from 0.1 to 5.0 %), it can be disrupted not only mechanically, but a part of monosaccharides contained therein can be hydrolysed as well. Tengborga et al. [6] managed to get 80% of hemi-cellulose and 8 % of glucose from the absolute output, during the hydrolysis process, when the technological parameters were as follows: the biomass retention temperature in the reactor was 180 °C, the duration of the process – 20 minutes and the concentration of sulphuric acid in biomass – 0.5 %. Nguenas et al. [7] received 79 % of hemi-cellulose and 21 % of glucose output from the absolute output, when the temperature in the reactor was 180 °C, the process duration – 4 minutes, and the concentration of sulphuric acid in biomass – 2.66 %.

The formation of inhibitors was the main reason which limited the increase of the temperature in the reactor, concentration of sulphuric acid in biomass and the lengthening of the duration of hydrolysis, i.e. the formation of compounds which decelerated the enzyme operation during fermentation process. The main inhibitors were furfural, received from xylose, exposed to high temperature and catalytic acid 5-hydroxymethylfurfural (HMF), and produced from glucose under the same conditions. Sanchez et al. investigated the furfural impact on the fermentation process and determined that 0.2 % of furfural concentration stopped the enzyme operation completely [8]. Taherzadeh et. al. [9] disclosed that 2 g/l of HMF increased the ethanol output insignificantly but reduced the micro organism productivity by 20 %. Martin and Jonson [10] maintain that the amounts of furfural and HMF during fermentation should not exceed 0.7 and 0.95 g/l. Raw materials such as wood of deciduous and softwood trees,

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maize stems and straw used in bioethanol production were exposed to high pressure steam [11-16].

There were not found the research data on crops, when the whole plant (i.e. both the stem and grains at the same time) was exposed to high pressure steam. If compared with previously mentioned materials, crops, as raw materials for bioethanol production, have many advantages:

- Combines for harvesting are not necessary;
- High biomass energy content per unit volume;
- Well-developed growing technologies.

The studies have not determined yet what technological parameters of pre-treatment hydrolysis (steam pressure, temperature, retention duration in reactor, the amount of catalytic acid) are needed, that the fibrous structure of crops would be disarranged and most of the useful monosaccharides (glucose, arabinose, xylose and mannose) which are necessary in later processes of bioethanol production would be extracted.

The Aim of Work—To investigate the process of pre-treatment hydrolysis of rye and triticale in bioethanol production, to justify the main technological parameters of pre-treatment hydrolysis process and to determine the amounts of monosaccharides.

II. METHODOLOGY

A. Raw Material Preparation

The studies were carried out in the laboratories of Renewable Materials Technology of Wismar University of Technology, Business and Design in Germany. Rye (*Avanti*) and triticale (*Tricolor*) were being grown in the fields of Experimental Station of Wismar University of Technology, Business and Design. Yellow ripeness crops were being cut on July, whereas ripe crops were being cut manually on August. 20 kg of every species, as well as, of various maturity stages crops were cut and the samples were carried to the laboratory. In the laboratory, the samples were chopped with a special stand, consisting of the frame, 18 kW electric motor, case with cover, cutting shaft with 36 knives, sieve, ventilator and discharge duct. Crops of each species and various maturity stages were thrown into the hopper, from which, later on, were supplied into the cutting apparatus. The rotation frequency of the cutting shaft with knives was 2920 min^{-1} . The chopped crops (length of the chaff was from 2 to 15 mm) were falling through the sieve and were carried away via the discharge by the air flow of the pneumatic ventilator. The mass of the chopped crop was placed into the polyethylene bags. The chopped crop mass of every species and different maturity were divided into 40 smaller samples of 500 g each and placed into the polyethylene bags which, later on, were stored in the refrigerated cabinet at -2°C .

B. Hydrolysis Process

The samples of yellow ripeness rye were taken from refrigeration cabinet and each of them was mixed with 2 litres of distilled water or sulphuric acid solution and kept in the

room temperature for 10-12 hours, for distilled water to soak into the mass. What is more, later, each sample of the chopped rye and distilled water mass was placed into a high pressure reactor, one by one (Fig. 1).

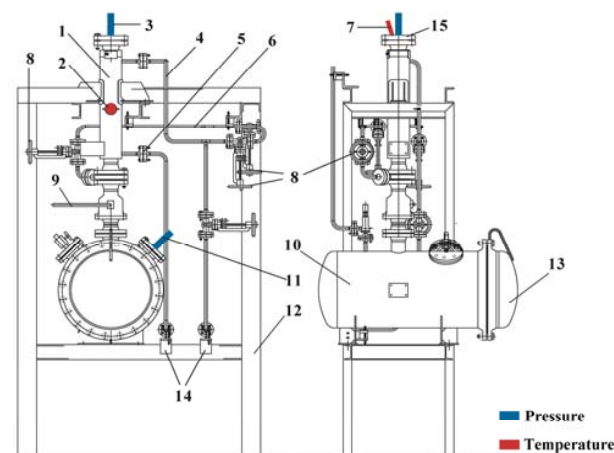


Fig. 1 Pre-treatment hydrolysis apparatus:

- 1 – Reactor; 2, 11 – Temperature sensors; 3 – Pressure sensor;
4, 6 – High pressure steam tubes; 5 – Steam condensate pot;
7 – Temperature sensor; 8 – Steam supply control valves; 9 – Main valve handle; 10 – Mass expansion vessel; 12 – Frame; 13 – Cover of mass expansion vessel; 14 – Condensate collection stoups;
15 – Reactor cover

The reactor (1) consisted of the cover (15) a vessel with two walls, inside of which - the perforated central tube was installed. Pressure (3) and temperature (2, 11) sensors were installed on the reactor. Via the tubes (4, 6), the reactor was connected with the equipment (18 kW electrical steam generator the permissible steam production capacity of which was 22 kg/h, pressure – 32 bar, and temperature – 238°C) supplying the high pressure steam, as well as, with the mass expansion vessel (10).

The sample of the chopped mass was placed around the perforated tube, located inside the reactor. The high pressure steam was being supplied between the reactor vessel walls, until they reached the temperature of 130°C . The wall temperature was fixated by temperature sensor (7). When the walls heated until 130°C , the high pressure steam (20-22 bars) was directed to the tube, located inside the reactor. The chopped mass heated until 210°C . The mass temperature was being measured by another temperature sensor (2). The chopped mass inside the reactor was being heated for 180-240 seconds. Under the high temperature influence, the connections between monosaccharides in the chain of hemicellulose were being broken. The acid was used as the catalyst for the reaction acceleration. Later on, the high pressure steam supply was stopped and by pressing the handle (9) the main valve is opened, with the help of which the pressure was quickly reduced to 2.5 bars. The heated mass, because of the sudden reduce in pressure, was being thrown from the reactor into the mass expansion vessel (10). When the pressure

reduced, the water inside the plant instantly evaporates and breaks the fibrous biomass structure.

When the cover of mass expansion vessel (13) had been opened, the disarranged the mass of the chopped rye fibre (hydrolysate) was collected into the bucket, which later on was used for the amounts of dry matters and monosaccharides to determine. The same tests were carried out with two more samples, when the chopped rye fibre of one of the samples was disarranged.

Experiments with other rye and triticale samples were carried out by changing the pressure, the time which the chopped mass was kept in the reactor and the amount of catalytic sulphuric acid in the distilled water (Table I). Each experiment was repeated 3 times.

TABLE I

PRESSURE AND TIME PARAMETERS, AMOUNTS OF CATALYTIC ACID DURING THE HYDROLYSIS OF RYE AND TRITICALE AT YELLOW RIPENESS AND RIPE

| THE HYDROLYSIS OF RYE AND TRITICALE AT YELLOW RIPENESS AND RIFE | | | | | | |
|---|--|----|-----|----|-----|----|
| Mass retention duration in the reactor, s | Amount of catalytic sulphuric acid in the distilled water, percent | | | | | |
| | 0 | | 0.3 | | 0.5 | |
| | Pressure in the reactor, bar | | | | | |
| | 20 | 22 | 20 | 22 | 20 | 22 |
| 180 | Rye at yellow ripeness and ripe Triticale at yellow ripeness and ripe | | | | | |
| 240 | Rye at yellow ripeness and ripe Triticale at yellow ripeness and ripe | | | | | |

Two samples of 200 g, were taken from the mass with disarranged fibrous structure (hydrolysate) of each sample, poured into glass container and kept in the drying cabinet for 7 days at the temperature of 60 °C. After 7 days, the samples were taken from the drying cabinet and placed into the hermetic cooling vessel for one hour. Then the samples were weighted and the amount of the dry matter was calculated.

C. Monosaccharide Extraction

The sample of 10-15 g was taken from the mass with disarranged fibrous structure (hydrolysate) which was taken out from the mass expansion vessel and placed into the flask, and the amounts of glucose, arabinose, xylose, mannose, galactose, furfural and HMF were identified in the chemical research laboratory with chromatograph GC-17A, with the column DB-225 made by Shimadzu company. The amount of various materials which was received with chromatograph was determined according to ASTM-E 1821-96 method (standard material determination method with gas chromatograph), and the recorded data was stored in the computer. Amounts of monosaccharide in all the samples of rye and triticale at yellow ripeness and ripe were defined using the same principle.

III. RESULTS

The pre-treatment hydrolysis studies showed that the mass of disarranged structure fibre of triticale at yellow ripeness (hydrolysate), which was disarranged while changing the amount of catalytic sulphuric acid from 0 until 0.5 %, the

steam supply pressure from 20 until 22 bar, and when the retention time in the reactor was 180 s, contained from 15.2 to 16.8 % of the dry matters (Table II).

TABLE II
DRY MASS CONTENT (IN PERCENT) IN RYE AT YELLOW RIPENESS
HYDROLYSATE

| Mass retention duration in the reactor, s | Amount of Catalytic sulphuric acid in the distilled water, percent | | | | | |
|---|--|-----------|-----------|-----------|-----------|-----------|
| | 0 | | 0.3 | | 0.5 | |
| | Pressure in the reactor, bar | | | | | |
| | 20 | 22 | 20 | 22 | 20 | 22 |
| 180 | 16.8 ±1.7 | 16.5 ±0.4 | 15.2 ±0.4 | 15.4 ±0.2 | 16.6 ±1.5 | 15.9 ±0.3 |
| 240 | 16.4 ±0.7 | 17.7 ±0.3 | 16.0 ±0.3 | 16.2 ±0.8 | 17.7 ±0.2 | 17.1 ±0.3 |

The influence of steam pressure supply and amount of catalytic acid on the amount of dry matter of rye at yellow ripeness was not significant. When the duration of mass retention in reactor was extended from 180 to 240 s, the amount of the dry matter was approximately 0.5 % larger.

The experiments of the triticale at ripe showed that when mass retention duration in the reactor was 180 s, approximately 8.8 % of the dry matters was received. The steam supply pressure and amount of catalytic sulphuric acid did not have any significant influence on the amount of the dry matter of the triticale at ripe. When mass retention duration in the reactor was extended by 240 s, averagely about 9.5 % of the dry matter was received.

When analogous studies were performed with triticale mass at yellow ripeness, there was determined that, when the mass retention duration in the reactor was 180 s, the amount of the dry matter in hydrolysate was from 15.7 to 18.3 %, whereas when the mass retention duration was 240 s – from 16.0 to 18.8 %. Moreover, when mass retention duration in the reactor was 180 s, the amount of the dry matter in triticale at ripe was from 11.1 to 12.6 %, whereas when mass retention duration was 240 s – from 11.8 to 12.6 %.

The data of the pre-treatment hydrolysis dry matter showed that the samples of rye and triticale at yellow ripeness, mixed with 2 litres of distilled water, in the reactor were disarranged worse than the samples of rye and triticale at ripe.

The monosaccharide extraction tests of disarranged rye mass (hydrolysate) at yellow ripeness showed that when the amount of sulphuric acid was 0 %, the pressure was 20 bar and mass retention was 180 s, 0.9 % of glucose, 0.35 % of arabinose, 0.15 % of xylose, and 0.5 % of mannose were received, if calculated from the dry matter. The undesirable materials, such as furfural and HMF were not formed (Fig. 2).

When retention duration in the reactor was expanded by 240 s, the amount of glucose increased in 0.5 %, whereas of other materials – not significantly. When the steam pressure was increased from 20 to 22 bar, the amount of glucose increased the most (by 3.4 % when the duration was 180 s and by 2.3 % when the duration was 240 s), whereas the amount of other materials – almost did not change.

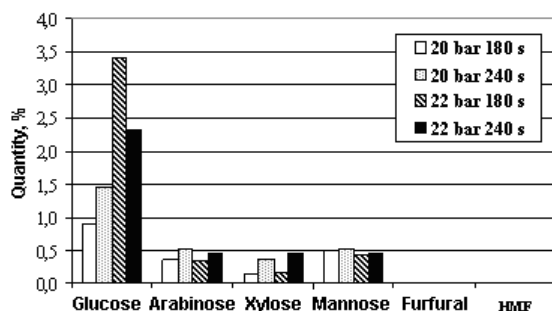


Fig. 2 Dependence of monosaccharides upon steam pressure, temperature and reaction time in hydrolysed rye at yellow ripeness without sulphuric acid

When samples of rye at yellow ripeness were mixed with 0.3 % of catalytic sulphuric acid, pre-treatment hydrolysis tests showed that the amounts of all materials in hydrolysate increased (from 2 to 6 times), if compared with the samples containing 0 % of sulphuric acid. The largest amount of glucose (6.9 %) formed in the samples, when pressure in the reactor was 22 bars and the retention time was 240 s. The amount of other materials in the hydrolysate was averagely from 0.7 to 2.0 %. The change of pressure and retention duration in the reactor did not have any significant influence on the amount of materials.

When catalytic sulphuric acid was used, the formation of all materials increased. Moreover, the undesirable materials, such as furfural and HMF formed as well (furfural – by 0.7 %, HMF – by 0.4 %). (Fig. 3).

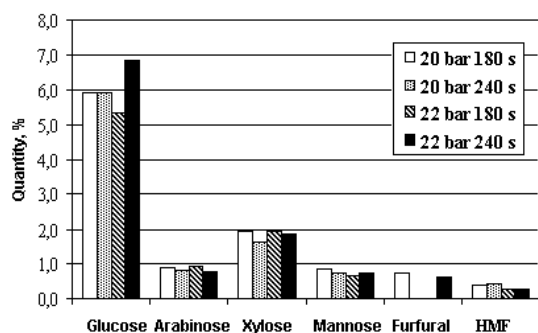


Fig. 3 Dependence of monosaccharides upon steam pressure, temperature and reaction time in hydrolysed rye in yellow ripeness with 0.3 % sulphuric acid

When the samples of rye at yellow ripeness were mixed with 0.5 % of catalytic sulphuric acid, the pre-treatment hydrolysis tests showed that, when the pressure was 20 bar and the retention duration was 180 s, the amounts of sulphuric acid in hydrolysate was 10 %, arabinose – 1.1 %, xylose – 2.9 %, and mannose – 0.9 %, whereas the amount of furfural – 0.5 %, whereas of HMF – 0.5 %. (Fig. 4). When retention duration in the reactor was expanded from 180 s to 240 s, and

the steam pressure was increased from 20 to 22 bars, the amount of all the materials changed insignificantly.

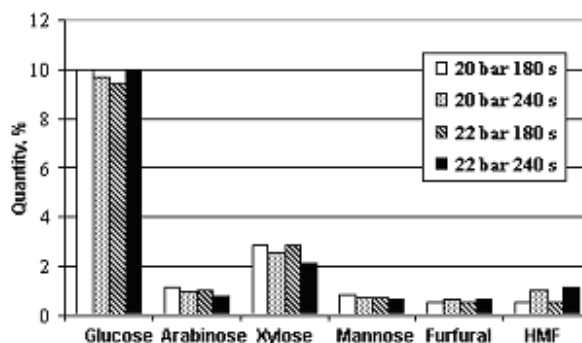


Fig. 4 Dependence of monosaccharides upon steam pressure, temperature and reaction time in hydrolysed rye in yellow ripeness with 0.5 % sulphuric acid

The studies of pre-treatment hydrolysis performed with the samples of the rye at ripe, which did not contain any catalytic sulphuric acid, showed that, when the pressure in the reactor was 20 bar and the retention time was 180 s, 1.8 % of glucose, 1.0 % of arabinose, and 0.5 % of xylose occurred, whereas other monosaccharide – did not occur (Fig. 5).

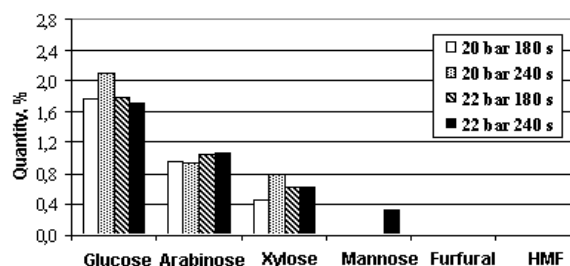


Fig. 5 Dependence of monosaccharides upon steam pressure, temperature and reaction time in hydrolysed ripe rye without sulphuric acid

When retention duration in the reactor was expanded to 240 s, the amount of glucose and xylose increased about 0.3 %, whereas other materials – did not form. When steam pressure was increased from 20 to 22 bar, about 1.8 % of glucose, 1.1 % of arabinose, and 0.6 % of xylose were determined, whereas when steam pressure was 22 bars and retention duration was 240 s, about 0.3 % of mannose formed as well.

When pre-treatment hydrolysis of the ripe and yellow ripeness were being compared, there was determined that in the hydrolytes at ripe, which were received when pressure was 20 bar, duration of retention – 180 s, and the amount of catalytic sulphuric acid – 0 %, the extraction of glucose was averagely about 2 times, arabinose – about 2 times, xylose – about 3 times larger than in the hydrolytes at yellow ripeness. When pressure was 22 bar, the glucose amount in hydrolytes was averagely 1.4 times smaller, whereas of other materials –

from 2 to 3 times larger than in the hydrolytes at yellow ripeness.

When the samples of rye at ripe were mixed with 0.3 % of catalytic sulphuric acid concentration, the pre-treatment hydrolysis tests showed that in hydro late, which was received in the reactor, when the pressure was 20 bar and the retention duration was 180 s, 6.8 % of glucose, 2.5 % of arabinose, 2.4 % of xylose, 1.1 %, and about 0.4 % of undesirable HMF formed (Fig. 6).

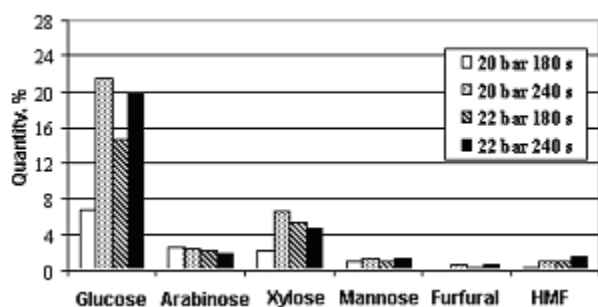


Fig. 6 Dependence of monosaccharides upon steam pressure, temperature and reaction time in hydrolysed ripe rye with 0.3 % sulphuric acid

When retention duration in the reactor was expanded by 240 s, about 3 times more of glucose and xylose formed, whereas the amount of other materials changed insignificantly. Moreover, undesirable furfural occurred as well (about 0.7 %). When steam pressure was increased in the reactor from 20 by 22 bar, the amount of glucose and xylose increased 2 times (180 s), whereas of arabinose and mannose – did not change. The amount of undesirable materials slightly increased.

When amount of catalytic sulphuric acid in rye samples at ripe was increased by 0.5 %, the extraction of all the good monosaccharide improved. The amount of glucose increased from 1.7 to 4.5 times, xylose – from 1.3 to 3.5 times, arabinose – about 1.5 times, whereas the amount of mannose and undesirable materials differed insignificantly, if compared with rye samples at ripe, containing 0.3 % of catalytic sulphuric acid. The most of monosaccharides were extracted when the pressure was 20 bars and retention duration was 240 s (Fig. 7).

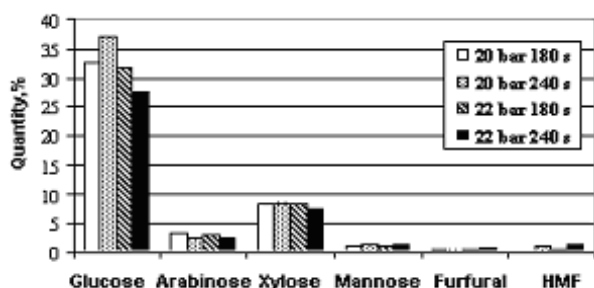


Fig. 7 Dependence of monosaccharides upon steam pressure, temperature and reaction time in hydrolysed ripe rye with 0.5 % sulphuric acid

When monosaccharide extraction from rye at ripe and yellow ripeness were being compared, there was determined that when rye samples at ripe contain 0.5 % of catalytic sulphuric acid, the amounts of glucose, arabinose and xylose increased about 3 times, of mannose – about 2 times, whereas the amount of the undesirable materials (furfural and HMF) increased insignificantly, if compared with rye samples at yellow ripeness.

Analogous studies were performed with triticale as well. If compared with the data of pre-treatment hydrolysis studies of rye at yellow ripeness and ripe, in the triticale hydrolysate at yellow ripeness and ripe, the amount of the dry matter was about 1.5-2 % larger.

In triticale hydrolysate at yellow ripeness, which was received not using any of catalytic sulphuric acid (0 %), there was determined that, when pressure was 20 bar and retention duration in the reactor was 240 s, the amount of glucose was about 40 %, and of other monosaccharide – about 2 times smaller, if compared with rye at yellow ripeness.

When the catalytic sulphuric acid concentration in triticale samples at yellow ripeness was increased by 0.5 %, the pre-treatment hydrolysis process showed that, the amount of all monosaccharide was similar as in the studies, where sulphuric acid concentration was 0.3 %, steam pressure – 22 bar, and retention duration in the reactor – 180 s. Furthermore, studies of triticale at yellow ripeness showed the further increase in the catalytic sulphuric acid concentration was not necessary, since the amount of monosaccharide increased insignificantly, whereas of the undesirable materials – more rapidly.

The comparison of the best results of rye and triticale monosaccharide extraction at yellow ripeness showed, that about 30 % of glucose and about 2 times more of other materials were received from rye at yellow ripeness than from the triticale at yellow ripeness.

The pre-treatment hydrolysis and monosaccharide extraction studies determined that most of the useful monosaccharide can be received from the rye at ripe, the sample of which were mixed with 0.5 % concentration of catalytic sulphuric acid, and were hydrolysed in the reactor, where the pressure was 20 bar and retention duration was 240 s. The most of undesirable materials were extracted from rye at ripe, when the sulphuric acid concentration was 0.3 %, the pressure – 22 bar and retention duration – 240 s.

IV. CONCLUSION

The intensity of the disarrangement of fibrous structure of grain mass and the amount of monosaccharide and unacceptable materials change, when the strength of the catalytic sulphuric acid (from 0 to 0.5 %), steam pressure (from 20 to 22 bar), and the reaction time in the reactor (from 180 to 240 s) are being changed.

The largest amount of the green matter was found in the hydrolysate of triticale at yellow ripeness (18.8 %), when the sulphuric acid concentration was 0.5 %, steam pressure 20 bars and retention time in reactor 240 s. In order to disarrange

the samples of rye and triticale at ripe better, from 1.5 to 2 times less of the dry matter was determined, if compared with the samples at yellow ripeness.

Most of monosaccharide (37.2 % of glucose, 2.7 % of arabinose, 8.4 % of xylose, and 1.3 % of mannose) was received from rye at ripe, the samples of which were mixed with 0.5 % concentration of the catalytic sulphuric acid, and hydrolysed in the reactor, where the pressure was 20 bars and retention duration was 240 s.

REFERENCES

- [1] C. Berg, "World Ethanol Production", www.distill.com/world_ethanol_production.html. 2012.
- [2] M. Roehr, "The Biotechnology of Ethanol", Classical and Future Applications. Weinheim, 2001, 232 p.
- [3] J. N. Saddler, L. P. Ramos and C. Breul, "Stream Pretreatment of Lignocellulosic Residues", Bioconversion of Forests and Agricultural Plant Residues. 1993, pp. 73-91.
- [4] H. H. Dietrich, M. Sinner and J. Puls, "Potential of steaming hardwoods and straw for feed and food production", *Holzforschung*. 1998, vol. 32, pp. 193-199.
- [5] R. H. Marchessault, S. Coulombe, T. Hanai and H. Morikawa, "Monomers and oligomers from wood", *Pulp and Paper Magazine of Canada Trans*. 1980, vol. 6, pp. 52-56.
- [6] C. Tengborg, K. Stenberg, M. Galbe, G. Zacchi, S. Larsson, E. Palmqvist and B. Hahn-Hägerdal, "Comparison of SO₂ and H₂SO₄ impregnation of softwood prior to steam pretreatment on ethanol production", *Applied Biochemistry and Biotechnology*. 1998, vol. 70-72, pp. 3-15.
- [7] Q. A. Nguyen, M. P. Tucker, F. A. Keller and F. P. Eddy, "Two-stage dilute acid pretreatment of softwoods", *Applied Biochemistry and Biotechnology*. 2000, vol. 84-86, pp. 561-576.
- [8] B. Sanchez and J. Bautista, "Effects of Furfural and 5-hydroxymethylfurfural on the Fermentation of *Saccharomyces cerevisiae* and Biomass Production from *Candida guilliermondii*", *Enzyme Microbial Technology*. 1988, vol. 10, pp. 315-318.
- [9] M. J. Taherzadeh, L. Gustafsson, C. Niklasson and G. Liden, "Physiological effects of 5-hydroxymethyl furfural (HMF) on *Saccharomyces cerevisiae*", *Applied Microbiology and Biotechnology*. 2000, vol. 53, no 6, pp. 701-708.
- [10] C. Martín and L. J. Jönsson, "Comparison of the resistance of industrial and laboratory strains of *Saccharomyces* and *Zygosaccharomyces* to lignocellulose-derived fermentation inhibitors", *Enzyme Microbial Technology*. 2003, vol. 32, pp. 386-395.
- [11] J. Puls, K. Poutanen, H. Korner and L. Viikari, "Biotechnical utilisation of wood carbohydrates after steaming pre-treatment", *Applied Microbiology and Biotechnology*. 1985, vol. 22, pp. 416-423.
- [12] W. Schwald, T. Smaridge, M. Chan, C. Breuil and J. N. Saddler, "The influence of SO₂ impregnation and Fractionation on product recovery and enzymic hydrolysis of steam-treated sprucewood", In Coughlan, M. P. *Enzyme systems for lignocellulose degradation*. Elsevier Applied Science, London and New York. 1989, pp. 231-242.
- [13] L. Petrus and N. A. Noordermeer, "Biomass to biofuels, a chemical perspective", *Green Chemistry*. 2008, vol. 8, pp. 861-867.
- [14] R. Marchal, M. Ropars and J. P. Vandecasteele, "Conversion into acetone and butanol of lignocellulosic substrates pretreated by steam explosion", *Biotechnology Letters*. 1986, vol. 8, pp. 365-370.
- [15] I. E. Tenrud, O. Theander, L. Torneport and L. Vallander, "Changes in the chemical composition of steam-exploded wheat straw during enzymic hydrolysis", *Enzyme and Microbial Technology*. 1989, vol. 11, pp. 500-506.
- [16] P. Dürre, "Biobutanol: an attractive biofuel", *Biotechnology Journal*. 2007, vol. 2, no 12, pp. 1525-1534.