Annals of Warsaw University of Life Sciences – SGGW
Forestry and Wood Technology No. 110; 92-96
(Ann. WULS–SGGW, For. and Wood Technol. 110, 2020)

The influence of urea and formaldehyde on enzymatic hydrolysis of cellulose

MONIKA MARCHWICKA, ANNA LESIAK, ANDRZEJ RADOMSKI
Department of Wood Science and Wood Protection, Warsaw University of Life Science - SGGW

Abstract: Effect of selected urea and formaldehyde concentrations on glucose yield of enzymatic hydrolysis of cellulose was investigated. Urea and formaldehyde were added separately at the concentrations of 0.001, 0.002 and 0.005 g/cm^3. Glucose was determined by high-performance liquid chromatography (HPLC). It was found that the used concentrations of urea didn't influence glucose yield. In the case of formaldehyde, the results vary between used concentrations. The glucose yield of enzymatic hydrolysis of cellulose with the highest investigated concentration of formaldehyde (0.005 g/cm^3) decreased by 50 %.

Keywords: enzymatic hydrolysis of cellulose, inhibitors, urea, formaldehyde

INTRODUCTION

The wood industry generates plenty of wastes, among them post-consumer materials. Very common post-consumer wood-based material is particle board glued with urea-formaldehyde (UF) resin (Mabee and Saddler 2010, GUS 2020). Utilization costs are high. Therefore, the most favourable way to solve the problem would be to find recipients who will use this material for their purpose. This would not generate additional costs and even could generate profits (Cheremisinoff 2002).

The possibility of converting wood biomass from post-consumer boards into biofuel gives a chance to find material that is not used in other industries and also prevent causing environmental burdens such as waste storage or the release of harmful compounds in the decomposition process or combustion one (Jafari et al. 2011). Poland is a country potentially rich in biomass, in the form of post-consumer wood materials (GUS 2020).

Also, an important reason to consider and investigate the usage of post-consumer materials for bioethanol production is a fact that European Union countries are constantly obliged by directives and other legal acts to ensure a minimum share of renewable energy in final energy consumption, e.g. Directive (EU) 2015/1513. It also includes an increase in the use of fuels produced from the raw materials listed in the legal acts, such as inedible lignocellulosic materials. The geographical location of Poland causes the need to look for green energy sources beyond solar, wind or water. Currently, the focus for energy production is on the plant biomass (Möller 2006; Drapcho et al. 2008; Hamelinck et al. 2005). Therefore, usage of post-consumer materials would allow to partially meet the market demand for source material for biofuel production without depleting higher quality raw materials that can be used in other industries (e.g. paper, furniture, wood-based panels).

Particleboard is made of wood which lignocellulosic structure is formed by cellulose, hemicelluloses (a potential source of fermentable sugars) and lignin. It is known as a lignin-carbohydrate complex (LCC) in which carbohydrate polymers are tightly bound to the aromatic polymer. Because most microbes are not able to degrade the LCC, it is considered unfermentable in its native form. Despite the significant potential of lignocellulosic biomass, some challenges are to overcome (Thomas and Kwang 2001). To make the LCC fermentable material simple sugars must be extracted from it. One way to break down cellulose to glucose is hydrolysis catalyzed by cellulolytic enzymes. To enhance glucose yield of wood material it is necessary to make cellulose more accessible. For lignocellulosic biomass, pretreatment that improves the subsequent stages and the overall efficiency of the bioethanol technology is applied (Modenbach and Nokes 2013; Prasad et al. 2019).

There are many pretreatment methods use for lignocellulosic materials. The most popular of them are physicochemical, such as steam explosion (SE) or liquid hot water (LHW), include thermohydrolytic treatment. Effect of hydrolysis in acid and alkaline pH at different temperatures on UF resin degradation was investigated, among them Dutkiewicz (1983), and it showed that the amount of insoluble fraction of resin decreased with the temperature rise. Hydrolysis of UF resins in acid and alkaline pH caused a formaldehyde release. Also, during thermohydrolysis of particleboards glued wit UF resin formaldehyde is released - noticeable amounts at 40 °C after 24 h, maximum at 105 °C after 24 h (Roffael and Hüster, 2011).

During the pretreatment of particleboard with UF resin, there are many changes, one of them is releasing some amounts of aldehyde and urea from UF resin. This investigation aimed to check if this single factor – the presence of urea or formaldehyde - affect the enzymatic hydrolysis yield. To see it clearly and without any other factors that can influence the enzymatic hydrolysis, it was performed on cellulose. In this work, it was investigated if the selected concentrations of UF resin ingredients inhibit the glucose yield of enzymatic hydrolysis of cellulose with the usage of industrial enzymes.

MATERIALS AND METHODS

The cellulose used in this work was Whatman® Qualitative Filter Paper Sheet. Cellic® CTec2 industrial enzymes from Novozymes were used for cellulose hydrolysis. It is a specialized cellulase complex designed to degrade cellulose to glucose. The mixture includes cellulases (endo and exoglucanases), β-glucosidase and hemicellulases (the manufacturer does not provide information about individual enzymes) (Novozymes 2010).

For enzymatic hydrolysis, 100 mg of cellulose dry matter was weighed. The material was placed in a 10 cm^3 glass tube. Citrate buffer pH 4.8, 0.1 M sodium azide solution, 25 % enzyme solution and distilled water were added to the tube to the total volume. The exact amounts of ingredients are shown in Table 1.

The prepared and hermetically sealed mixture of components for enzymatic hydrolysis was placed in an oven at 50 °C. The enzymatic hydrolysis was carried out in an ELMI rotator model Intelli-mixer RM-2 at speed 25 rpm. After 72 hours of enzymatic hydrolysis, samples were collected and stored frozen at –24 °C until HPLC analysis.

Tab. 1. Volume and mass of the components of the enzymatic hydrolysis mixture.

Total volume /cm^3	10
Cellulose mass /mg	100
Enzyme /mg	100
Buffer /cm^3	5
0.1 M sodium azide solution /cm^3	0.1
Distilled water /cm^3	up to 10

Enzymatic hydrolysis of cellulose was carried out with and without the addition of formaldehyde and urea.

The resin makes up about 10 % of the board weight. The minimum used amounts of formaldehyde were chosen as if the same mass of formaldehyde was obtained from the resin as a result of the degradation. Two and five times more were also used. The amount of urea was calculated in the same way. To the test tubes, formaldehyde at concentrations of 0.001, 0.002 and 0.005 g/cm^3 was added as 40 % water solution (formalin). Urea was added at the same concentrations as above.

The obtained hydrolysates were analyzed using high-performance liquid chromatography (HPLC) to determine the glucose content. A column dedicated to the analysis of simple sugars, organic acids and alcohols (Rezex RHM-Monosaccharide H+ from Phenomenex) was used. Analysis conditions: flow 0.6 cm^3/min, temperature 80 °C, eluent redistilled water.

The enzymatic hydrolysis efficiency was determined by relating the obtained amount of glucose in the hydrolysate to the calculated theoretical glucose content (TGC). TGC was calculated based on the known cellulose mass of the sample, which was multiplied by a factor of 1.11 - corresponding to the ratio of glucose mass ($C_6H_{12}O_6$ – 180.16 u) to the mass of the glucopyranose residue ($C_6H_{10}O_5$ – 162.14 u).

RESULTS

Enzymatic hydrolysis was performed on cellulose with the addition of potential inhibitors formed during the pretreatment of the board (other than in the case of pretreatment of wood without resin) - formaldehyde and urea. For comparative purposes, the efficiency of enzymatic hydrolysis of cellulose without the addition of inhibitors was carried out. The results are presented in the chart below (Figure 1).

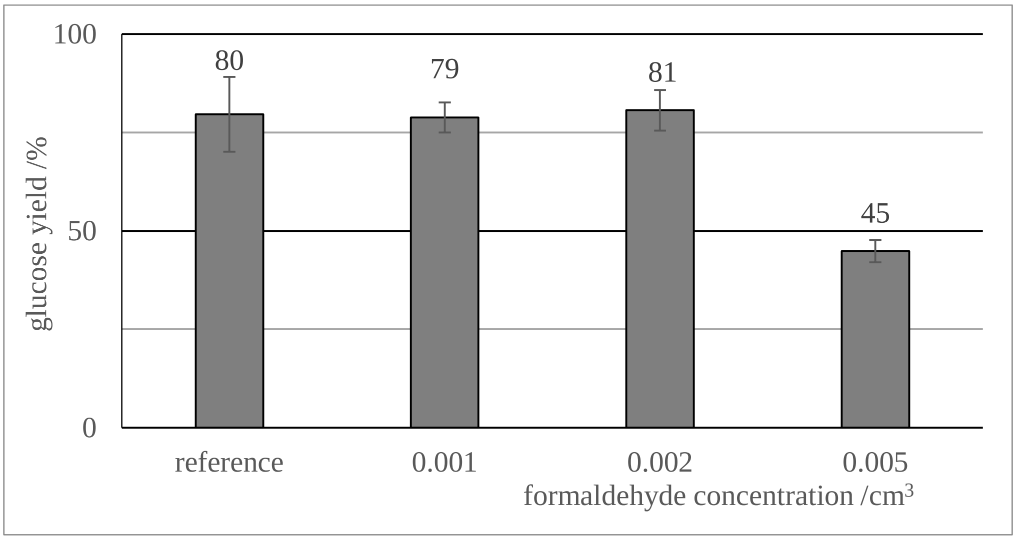

Fig. 1. Effect of formaldehyde on enzymatic hydrolysis of cellulose after 72 hours of the process.

Comparing the obtained glucose yield of enzymatic hydrolysis of cellulose with addition of formaldehyde to glucose yield of reference enzymatic hydrolysis of cellulose, the inhibitory effect of formaldehyde was found only for the concentration of 0.005 g/cm^3 – concentration five times greater than this potentially obtainable during the pretreatment process. The glucose yield of enzymatic hydrolysis with formaldehyde concentration of 0.005 g/cm^3 compared to reference enzymatic hydrolysis of cellulose decreased from 80 to 45 %. It was found that the concentrations of 0.001 and 0.002 g/cm^3 formaldehyde didn't affect the efficiency of enzymatic hydrolysis of cellulose which was 79 and 81 %, respectively.

No significant differences were observed between the glucose yield of enzymatic hydrolysis of cellulose with the addition of urea and glucose yield of reference enzymatic hydrolysis of cellulose. The glucose yield of enzymatic hydrolysis with a urea concentration of 0.002 g/cm^3 compared to reference one slightly increased from 80 to 84 %. Lou et al. (2018) investigated the effect of urea on the enzymatic hydrolysis with the usage of Cellic CTec2 of eucalyptus pretreated by dilute acid. They found out that urea concentration below 60 g/L (which is equal to 0.06 g/cm^3). enhanced the efficiency of enzymatic hydrolysis while above this concentration caused a decrease.

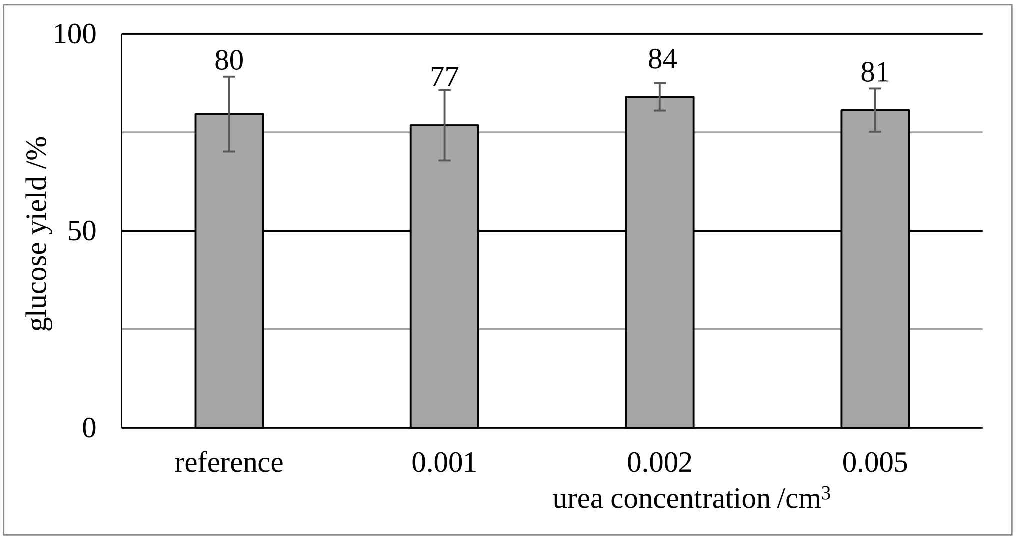

Fig. 2. Effect of urea on enzymatic hydrolysis of cellulose after 72 hours of the process.

They also found out that the addition of urea to enzymatic hydrolysis of microcrystalline cellulose (Avicel®) with Cellic CTec2 enzymes caused a decrease in efficiency of the process. In this work, no inhibitory effect of used concentrations of urea on glucose yield of enzymatic hydrolysis of cellulose was observed. The maximum concentration of urea applied in this investigation was 0.005 g/cm^3 which is strongly lower than this one used by Lou et al. (2018).

CONCLUSIONS

1. The concentration of 0.001 and 0.002 g/cm^3 of formaldehyde didn't inhibit the glucose yield after 72 h of enzymatic hydrolysis of cellulose.
2. The concentration of 0.005 g/cm^3 of formaldehyde inhibited the glucose yield after 72 h of enzymatic hydrolysis of cellulose to 45 %.
3. The concentration of 0.001, 0.002 and 0.005 g/cm^3 of urea didn't inhibit the glucose yield after 72 h of enzymatic hydrolysis of cellulose.

REFERENCES

1. Cheremisinoff N.P. (2002). Handbook of solid waste management and waste minimization technologies, Butterworth-Heinemann, Amsterdam
2. Directive (EU) 2015/1513 of the European Parliament and of the Council of 9 September 2015 amending Directive 98/70/EC relating to the quality of petrol and diesel fuels and amending Directive 2009/28/EC on the promotion of the use of energy from renewable sources
3. Drapcho C. M., Nhuan N.P., Walker T. H. (2008). Biofuels Engineering Process Technology, McGraw-Hill, USA
4. Dutkiewicz, J. (1983). Hydrolytic degradation of cured urea–formaldehyde resin. Journal of Applied Polymer Science, 28(11), 3313–3320
5. Gorczyca B. (ed.) (2020). GUS Statistical Yearbook of Industry-Poland 2019, Warsaw

https://stat.gov.pl/obszary-tematyczne/roczniki-statystyczne/roczniki-statystyczne/rocznik-statystyczny-przemyslu-2019,5,13.html
6. Hamelinck C.N., van Hooijdonk G., Faaij A.P.C. (2005). Future prospects for the production of ethanol from ligno-cellulosic biomass. Biomass & Bioenergy, 28, 4, 384-410
7. Jafari V., Labafzadeh S.R., Jeihanipour A., Karimi K., Taherzadeh M.J. (2011). Construction and demolition lignocellulosic wastes to bioethanol, Renew Energy, 36, 2771-2775
8. Lou H., Lin M., Zeng M., Cai C., Pang Y., Yang D., Qiu X. (2018). Effect of urea on the enzymatic hydrolysis of lignocellulosic substrate and its mechanism, Bioenergy Res., 11, 456-465
9. Mabee W.E., Saddler J.N. (2010). Bioethanol from lignocellulosics: status and perspectives in Canada, Bioresour. Technol., 101,11 4806-4813
10. Modenbach A.A., Nokes S.E. (2013). Enzymatic hydrolysis of biomass at high-solids loadings: A review. Biomass and bioenergy, 56, 526-44
11. Novozymes (2010). APPLICATION SHEET, Cellic® CTec2 and HTec2 – enzymes for hydrolysis of lignocellulosic materials
12. Prasad R.K., Chatterjee S., Mazumder P.B., Gupta S.K., Sharma S., Vairale M.G., Datta S., Dwivedi S.K., Gupta D.K. (2019). Bioethanol production from waste lignocelluloses: A review on microbial degradation potential. Chemosphere, 231, 588-606
13. Roffael E., Hüster H.-G. (2011). Complex chemical interactions on thermo hydrolytic degradation of urea formaldehyde resins (UF-resins) in recycling UF-bonded boards. European Journal of Wood and Wood Products, 70(4), 401–405
14. Thomas V.M., Kwong A. (2001). Ethanol as a lead replacement: phasing out leaded gasoline in Africa. Energy Policy, 29, 1133-1143

Streszczenie: *Wpływ mocznika i formaldehydu na hydrolizę enzymatyczną celulozy.* Prezentowane badania miały na celu określenie wpływu wybranych dodatków mocznika i formaldehydu na wydajność glukozy w enzymatycznej hydrolizie celulozy. Mocznik oraz formaldehyd dodano osobno w stężeniach 0,001, 0,002 i 0,005 g/cm^3. Wydajność glukozy po hydrolizie enzymatycznej oznaczano za pomocą wysokosprawnej chromatografii cieczowej (HPLC). Stwierdzono, że zastosowane stężenia mocznika nie miały wpływu na wydajność glukozy. W przypadku formaldehydu wyniki różnią się między zastosowanymi stężeniami. Wydajność glukozy hydrolizy enzymatycznej celulozy z największym badanym stężeniem formaldehydu (0,005 g/cm^3) była prawie o połowę mniejsza w porównaniu do hydrolizy enzymatycznej celulozy bez formaldehydu.

Corresponding author:
Monika Marchwicka,
Department of Wood Science and Wood Protection,
Warsaw University of Life Science- SGGW,
ul. Nowoursynowska 159
02-117 Warsaw, Poland
e-mail: monika_marchwicka@sggw.edu.pl

Investigations supported by a research project in the internal competition mode at Warsaw University of Life Sciences for a young researcher and PhD student in the year 2017 Investigation of the chemical properties of lignocellulosic biomass after the liquid hot water (LHW) pretreatment of wood chips manufactured with urea-formaldehyde resin in the context of the use of post-wood based materials in the biofuels sector” awarded to Anna Lewandowska.