Annals of Warsaw University of Life Sciences – SGGW
Forestry and Wood Technology № 112, 2020: 79-84
(Ann. WULS - SGGW, For. and Wood Technol. 112, 2020: 79-84)

# Change in hydrolytic enzyme efficiency over time

DOMINIKA SZADKOWSKA
Warsaw University of Life Sciences-SGGW, Department of Wood Science and Wood Preservation, Institute of Wood Sciences and Furniture, Poland

**Abstract:** *Change in hydrolytic enzyme efficiency over time*. The purpose of this study was to determine the action of hydrolytic enzymes (by Dyadic Cellulase CP CONC, and the Dyadic Xylanase 2 XP CONC) over time. Chromatographic analysis of holocellulose samples subjected to enzymatic hydrolysis was performed. The following hydrolysis parameters were used: time 48h, temperature 45 ºC, acetate buffer pH 5.4, commercial enzymes Dyadic. Holocellulose extracted by the sodium chlorite method from white poplar wood (*Populus alba* L.) was used. The final yield of enzymatic hydrolysis was determined. The results of hydrolysis performed at intervals were compared. The results obtained show that the hydrolysis yield of holocellulose after five months decreased by 40 p.p. for glucose yield and by 25 p.p. for xylose yield. The yield for glucose after two and a half years decreases by 68 p.p. and 62 p.p. for xylose compared to the initial yield.

**Keywords**: enzymatic hydrolysis, enzyme, poplar wood, holocellulose

## INTRODUCTION

During the 20th century, the global economy faced a huge problem in the form of crises in the availability of raw materials and the greenhouse effect, the impact of which showed itself with full intensity. Global organizations formed after the great conflicts of the 19th and 20th centuries (WHO, UN, Red Cross and Red Crescent) in order to counteract regional problems such as famine, epidemics, genocides or armed conflicts were forced to develop policies to counteract the impact of the greenhouse effect on the entire earth and to try to develop new energy technologies that can significantly reduce the use of fossil fuels. As a result of inter-governmental agreements and negotiations, a number of safeguards and regulations were created to combat climate change and reduce the large-scale use of fossil fuels.

As one of the ways to reduce the impact of the global economy on the earth's climate, the so-called carbon footprint of products is being addressed. To this end, a directive on responsible consumption and production by the United Nations Sustainable Development Goal 12 (SDG 12) (McCallum et al. 2021). In the outlined assumptions, one of the key elements is the use of lignocellulosic biomass as a substrate for the production of biofuels and other compounds needed to develop a sustainable global economy. The use of biomass can significantly reduce the carbon footprint and reduce the effects of global climate change (Ragauskas et al. 2006).An important additional asset in the fight against global warming is the introduction of so-called "green chemistry" as part of the sustainable growth economy policy. The creation of green chemistry technology requires a series of research and development of related technologies for the time being. But for now, one of its foundations is the large-scale use of enzymes in technological processes. Apart from high prices of enzymes used for specific process tasks. Their use affects the reduction of energy consumption of chemical processes and increases their efficiency. At the same time decreasing the amount of waste produced, which in addition can be easier to recycle. As a result, enzymes are already used in laundry detergents, for example (Tarczykowska et al. 2017).

Enzymatic hydrolysis is conversion of macromolecular polysaccharides to simple sugars, occurring under the influence of protein catalysts - enzymes (Hames et al. 2001). Enzymes are produced by living organisms. Their action is precisely defined and programmed by ribonucleic acids (RNA) of the organisms or cells that produce them. The development of enzyme technology and enzyme-mediated processes is making new applications of enzymes on

an industrial scale possible due to intensive research. Biotechnology makes it possible to develop enzymes or enzyme analogs with specific properties or applications. Successive generations of enzymes are characterized by higher efficiency and resistance to specific environmental factors. Unfortunately, in comparison to conventional chemical compounds, enzymes and their analogs are characterized by a certain shelf life after which they become denatured. This process is inevitable and irreversible but it occurs gradually leading to a gradual decrease in enzyme efficiency (Baweja et al. 2016).

Studies on global enzyme sales indicate that 31% are enzymes for food purposes (mainly starch processing and baking), 6% are feed enzymes, and the rest are technical enzymes (production of detergents, textiles, biofuels) (Berka and Chery 2006).

The selection of enzymes for industrial processing of polysaccharides is aimed at obtaining the most beneficial achievements in their application. The requirements for enzymes for industrial purposes are considered to be relatively high (Goodenough 1995). The main problems of using enzymes in biofuel industry are their high cost and often difficult availability of catalysts with well-defined properties. Enzymes act only on certain substrates, under specific conditions (pH, temperature, presence of inhibitors). Unfortunately, enzymes have characteristics that are not desirable in industry, such as: high solubility, instability and susceptibility to inhibition, inhibition by substrates or reaction products, high price due to complicated isolation and purification. The implementation of enzymes as industrial catalysts is an interdisciplinary task and requires the selection of enzymes with suitable properties, their improvement by biomolecular and physicochemical techniques, and the adaptation of industrial reactors for their use (Robertson, Steer 2004, Betancor et al. 2003).

From the perspective of modern biofuel technologies, enzymes are compounds that allow the extraction of simple sugars from lignocellulosic materials without leading to the formation of inhibitory compounds such as: furfural or hydroxymethylfurfural (Verardi et al. 2012). Additionally, they do not lead to the corrosion and destruction of industrial equipment (Leja et al. 2009). The used enzyme (the enzyme undergoes aging and protein denaturation) can be used in other applications such as high-protein pet food. The use of enzymatic hydrolysis in the biofuel production process is currently expensive due to high enzyme prices (So and Brown 1999, Mussatto et al. 2008, Paulová et al. 2013). However, these prices are steadily decreasing and the process does not produce chemical waste that is difficult to dispose of (Walker and Wilson 1991).

## MATERIALS

Holocellulose for this study was obtained according to the sodium chlorite method (Krutul 2002). Enzymatic hydrolysis was carried out on wet holocellulose. The moisture content of the material before hydrolysis was determined on independent samples according to Krutul 2002. A single dose of 1.7 g of dry holocellulose was used for hydrolysis. Hydrolysis was carried out at 45 ºC, with pH 5,4 obtained using acetate buffer.

Two enzymes were used to carry out the hydrolysis - in the first case Dyadic Cellulase CP CONC, in the second Dyadic Xylanase 2 XP CONC. The enzyme was stored in the fridge at 4-6 ºC. The safety data sheet for both enzymes does not provide an expiration date for the product, and how they lose they activity, when they are storage in the fridge. There is only information about storage in 25 ºC. The enzyme was in powdered form, before use it was dispersed in water at 25 ºC with stirring on a magnetic stirrer (250 rpm).

The analysis was carried out on the liquid chromatograph (Shimadzu Company, Kyoto, Japan) connected to the refractometric detector RID-10 A. In chromatography was used the Phenomenex Luna 5µ $NH_2$ 100Å column (Phenomenex, Torrance, USA). Samples were

analyzed with the dedicated program LC Solution v.1.21 SP1. The temperature of analysis was 50 °C, eluent flow 1,5 $cm^3$/min and eluent acetonitrile: water (80:20 v:v), sample volume 20 µm. Each sample was 2 times injected, and the values were averaged. Results were processed by using LC Solution v.1.21 SP1 software. For the quantitative determination of monosaccharides in hydrolysates, previously developed calibration curves were used. The calibration curve was made for five different concentration of used sugar standards. Sugars used as a standards was xylose (Chempur, Piekary Śląskie, Poland) and glucose (PoCH, Gliwice, Poland). The calibration curves developed to process are show in **Figure 1**.

glucose: y= 2E-0,9x; $R^2$=0,9996
xylose: y=1,7677E-08x; $R^2$=0,9996
**Figure 1**. Equations of the calibrations curves

RESULTS

Holocellulose extracted from white poplar wood was used in this study. Holocellulose was considered to be the wood-derived material with the simplest structure - where the influence of other wood components (lignin and extractives) will not be important for the hydrolysis process itself. The biggest problem in enzymatic hydrolysis of wood is the lignocellulosic structure of the substrate. Due to the limitation in the availability of polysaccharide chains for enzymes (Yosida et al. 2008). The commercial enzymes used in this work are mixtures of enzymes, building an enzyme complex capable of degrading lignocellulose. In Dyadic Cellulase CP CONC guaranteed activity is cellulase, side activity is Beta-Glucanase and dditional side activities is xylanase, pectinase, mannanase, xyloglucanase, laminarase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase, protease. On the other hand in Dyadic Xylanase 2 XP CONC the main activity is xylanase and the side activities are beta-glucanase and cellulase and the additional side activities are pectinase, mannanase, xyloglucanase, laminarase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase. In the

A comparison of enzyme efficiency depending on its storage time in refrigerated equipment was carried out.

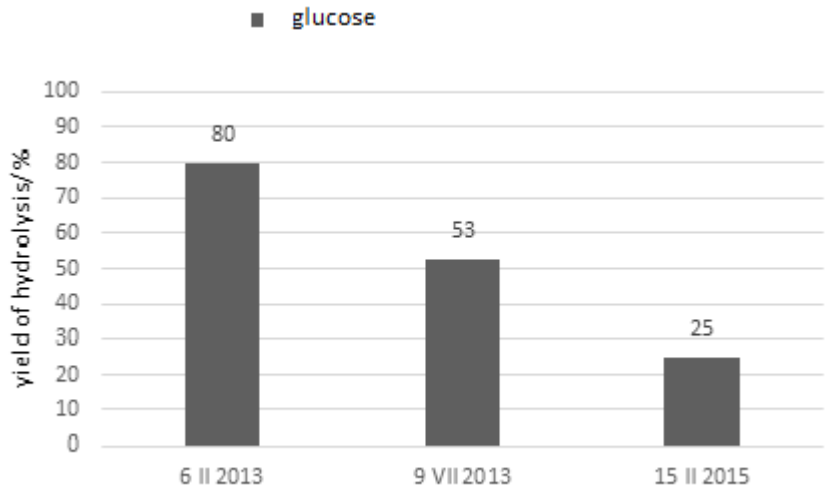


**Figure 2.** Comparison of the efficiency of the Dyadic Cellulase CP CONC enzyme

Based on the data in Fig. 2, it can be concluded that the highest hydrolysis efficiency for glucose at the most favorable conditions of the hydrolysis process (buffer pH and temperature) was obtained on 6 II 2013, and it was 80 %. After five months of enzyme storage, the efficiency of the process was 27 p.p. lower, and after 2.5 years it was 55 p.p. lower than first sample.

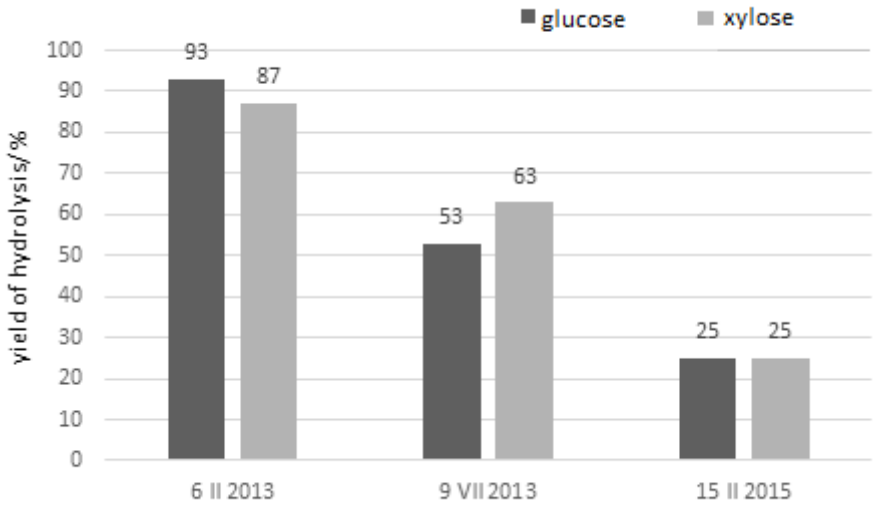


**Figure 3.** Comparison of effectiveness of Dyadic Xylanase 2XP CONC enzyme.

Using the Dyadic Xylanase 2XP CONC enzyme in the hydrolysis of holocellulose, the highest yield for the simple sugars tested was obtained in 6 II 2013 and was 93 % for glucose and 87 % for xylose (Fig. 3). After about five months of enzyme storage, the efficiency for glucose changed by 40 p.p. and for xylose by 25 p.p., and after 2.5 years by 68 p.p. and 62 p.p., respectively. The results obtained indicate a lower decrease in the efficiency of the enzymes responsible for xylose acquisition by hydrolysis.

The obtained results are consistent with the literature data, from which it is known that depending on the storage time of the enzyme, its activity in the hydrolysis processes of lignocellulosic raw materials decreases (Misset 1993).

## CONCLUSION

During storage, enzyme aging occurs, which is associated with a decrease in their activity in the hydrolysis of holocellulose, thereby affecting the reduction in hydrolysis efficiency toward glucose and xylose. The loss of enzyme performance after refrigerated storage is greater than the temperature reported in the data sheets.

The hydrolytic enzymes Dyadic Cellulase CP CONC, and Dyadic Xylanase 2 XP CONC lose their hydrolytic efficiency of holocellulose during storage. The decrease in enzyme efficiency due to refrigerated storage is greater than that stated by the manufacturer in the safety data sheet. The use of enzymes (Dyadic Cellulase CP CONC, and Dyadic Xylanase 2 XP CONC) that have been stored prior to their use in hydrolysis requires individual (depending on the storage time) selection of the amount of enzyme loads used in order to achieve optimal hydrolysis efficiency. Dyadic Cellulase CP CONC, and Dyadic Xylanase 2 XP CONC enzymes should be used as soon as possible after delivery from the manufacturer in order to minimise loss of efficiency.

REFERENCES


1. BERKA R.M., CHERRY J. R., 2006: Enzyme biotechnology. In Ratledge C, Kristiansen B, editors. Basic Biotechnology. 3rd edition. Cambridge, UK: Cambridge University Press,
2. BETANCOR L., A. HIDALGO, G. FERNÁNDEZ-LORENTE, C. MATEO, R. FERNÁNDEZ-LAFUENTE, J.M. GUISÁN, 2003: *Preparation of a stable biocatalyst of bovine liver catalase using immobilization and postimmobilization techniques,* Biotechnol. Prog. 19, 763-767.
3. Baweja M., Nain L., Kawarabayasi Y., Shukla P., 2016: *Current Technological Improvements in Enzymes toward Their Biotechnological Applications.*Front Microbiology 7, 1-13
4. GOODENOUGH P.W., 1995: *Molecular Biotechnology*, 4, 151-166.
5. HAMES B.D., HOOPER N.M., HOUGHTON J.D. 2001, Biochemia krótkie wykłady
6. KRUTUL D. 2002: Ćwiczenia z chemii drewna oraz wybranych zagadnień chemii organicznej. SGGW, Warszawa
7. LEJA K., LEWANDOWICZ G., GRAJEK W. 2009: *Produkcja bioetanolu z surowców celulozowych*. Biotechnologia 4(87), 88–101.
8. McCallum C.S., Wang W., Doran W.J., Forsythe W.G., Garrett M.D., Hardacre C., Leahy J.J., Morgan K., Shin D., Sheldrake G.N., 2021: *Life cycle thinking case study for catalytic wet air oxidation of lignin in bamboo biomass for vanillin production*. Green Chemistry, DOI: 10.1039/D0GC03685D
9. MISSET O.,1993: *Stability of Industrial Enzymes*, Studies in Organic Chemistry, 47, 111-113
10. MUSSATTO S.I. FERNANDES M., MILAGRES A.M.F., ROBERTO I.C. 2008: *Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain,* Enzyme and Microbial Technology 43,124-129
11. PAULOVÁ L., PATÁKOVÁ P., RYCHTERA M., MELZOCH K., 2013: *Production of 2nd Generation of Liquid Biofuels. w Liquid*, Gaseous and Solid Biofuels - Conversion Techniques pod red. Zhang Fang, 2
12. Ragauskas A.J., Williams C.K., Davison B.H., Britovsek G., Cairney J., Eckert C.A., Frederick W.J. Jr., Hallett J.P., Leak D.J., Liotta C.L., Mielenz J.R., Murphy R., Templer R., Tschaplinski T., 2006: *The Path Forward for Biofuels and Biomaterials.* Science 27, 484-489
13. ROBERTSON D.E., STEER B.A., 2004: *Recent progress in biocatalyst discovery and optimization,* Curr. Opin. Chem. Biol. 8, 141-149.
14. SO K. S., BROWN R. C. 1999: *Economic analysis of selected lignocellulose-to-ethanol conversion,* Technologies. Applied Biochemistry and Biotechnology, 77-79, 633, 640
15. VERARDI A., DE BARI I., RICCA E., CALABRO V., 2012: *Hydrolysis of lignocellulosic Biomass: Current Status of Processes and Technologies and Future Perspectives*. W Bioethanol pod red. M. A. Pinheiro Lima, 95-122
16. Tarczykowska A., Kochański B., Kałużny K., Zukow W., 2017: *Enzymes- important players in green chemistry*. Journal of Education, Health and Sport 7(9), 217-223
17. WALKER L.P., WILSON D.B., 1991: *Enzymatic hydrolysis of cellulose: An overiew*. Bioresource Technology, 36, 1, 3-14

18. YOSHIDA M., LIU Y., UCHIDA S., KAWARADA K., UKAGAMI Y., ICHINOSE M., KANEKO S., FUKUDA K. 2008: *Effects of Cellulose Crystallinity, Hemicellulose, and Ligninon the Enzymatic Hydrolysis of Miscanthussinensis to Monosaccharides*. Biosci. Biotechnol. Biochem., 72 (3), 805–810

Streszczenie. *Zmiana wydajności enzymów hydrolitycznych w czasie* Celem pracy było określenie działania enzymów hydrolitycznych w czasie. Przeprowadzono analizę chromatograficzną próbek holocelulozy poddanych hydrolizie enzymatycznej. Zastosowano następujące parametry hydrolizy: czas 48h, temperatura 45 ºC, bufor octanowy pH 5,4, enzymy komercyjne Dyadic. Zastosowano holocelulozę pozyskaną metodą chlorynu sodowego z drewna topoli bialej (*Populs alba* L.). Określono końcową wydajność hydrolizy enzymatycznej. Porównano wyniki hydrolizy przeprowadzonej w odstępach czasu. Uzyskane wyniki wskazują, że wydajność hydrolizy holocelulozy po pięciu miesiącach zmniejszyła się o 40 p.p. dla wydajności glukozy i o 25 p.p. dla wydajności ksylozy. Wydajność glukozy po dwóch i pół roku obniża się o 68 p.p., a ksylozy o 62 p.p. w stosunku do wydajności początkowej.

Corresponding author:

Dominika Szadkowska
Department of Wood Science and Wood Protection,
Instituite of Wood Sciences and Furniture
Warsaw University of Life Sciences (SGGW)
159 Nowoursynowska St., 02-776 Warsaw, Poland
e-mail: dominika_szadkowska@sggw.edu.pl