DOI:10.5937/FFR1902161C
UDK 577.152.3:577.112]:636.087.2

Original research paper

IMMOBILIZATION OF *ASPERGILLUS NIGER* CELLULASE ONTO LIFETECH™ CARRIERS AND ITS APPLICATION IN THE HYDROLYSIS OF SUNFLOWER SEED MEAL LIGNOCELLULOSIC FRACTION

Marija M. Ćorović*[1], Milica B. Simović[1], Ana D. Milivojević[2], Katarina M. Banjanac[2], Katarina D. Katić[3], Dejan I. Bezbradica[1]

[1] University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia
[2] University of Belgrade, Faculty of Technology and Metallurgy, Innovation Center, Karnegijeva 4, 11000 Belgrade, Serbia
[3] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia

*Corresponding author:
Phone: +381113303727
Fax: +381113370387
E-mail address: mstojanovic@tmf.bg.ac.rs

ABSTRACT: Cellulases are enzymes which catalyse cellulose hydrolysis and are widely used in various industry branches. Lately, their application in treatment of different agroindustrial waste materials which could serve for fuel production is being extensively explored. In order to increase their stability and cost-effectiveness of their usage, application of their immobilized forms are preferred over free enzymes. Hereby, we tested eight different Lifetech™ immobilization supports differing in polarity, porosity and functional groups as carriers for *Asspergillus niger* cellulase immobilization. Most promising carrier was methacrylate based, with primary amino groups, C6 "space arm" and pores with diameter of 60-120 nm - Lifetech™ ECR8409F. For this support, most important immobilization parameters were investigated and after 3 h at pH 6 with initial protein concentration of 23.3 mg/g support immobilized cellulase with 406 IU/g (with carboxymethyl cellulose as a substrate) was obtained. This preparation was successfully applied in the hydrolysis of lignocellulosic fraction of sunflower seed meal, which is widely available byproduct of sunflower seed meal fractionation for protein-rich fractions production. Initial reaction rates and yields of reducing sugars were unchanged comparing to free enzyme, indicating that there were no significant diffusion limitations for substrate to approach active sites of *A. niger* cellulase molecules immobilized onto Lifetech™ ECR8409F support.

Key words: *β-glucanase, immobilization, sunflower seed meal, lignocellulose, saccharification*

INTRODUCTION

Lignocellulosic (LC) materials are very abundant in nature and are also generated as waste byproducts of agro-industry. Due to its low price, availability and renewable character, lignocellulosic biomass is currently considered as a potential source of energy on large scales (Howard, Abotsi et al. 2003; Jørgensen, Kristensen et al. 2007; Kumar, Singh et al. 2008). In order to convert lignocellulose rich substrates into products with high added value such as biofuels and fine chemicals, multi-step processes are necessary – thermal, mechanical, chemical or biological pretreatment followed by enzymatic hydrolysis and microbial fermentation (Ji, Pang et al. 2008).

Cellulases are enzymes responsible for cellulose hydrolysis and are comprised of

catalysts demonstrating endo-(1,4)-*β*-D-glucanase (EC 3.2.1.4.), exo-(1,4)-*β*-D-glucanase (EC 3.2.1.91) and *β*-glucosidase (EC 3.2.1.21) activity (Juturu and Wu 2014). At this moment, cellulases are being applied in various industry branches such as agriculture, food, pharmaceutic, textile, paper and pulp (Kuhad, Gupta et al. 2011). Potential for their cost-effective application in the treatment of lingo-cellulosic biomass for the production of second generation biofuels is nowadays attracting increasing attention of scientific community. However, relatively high price of available enzyme preparations combined with high dosages required for efficient cellulose hydrolysis represents one of the main challenges for process commercialization. Convenient method for overcoming these issues is immobilization on adequate solid supports, providing increased operational and storage stability and reusability (Vaz, de Souza Moreira et al. 2016). Recently, microbial cellulases were immobilized onto different carriers, including magnetic chitosan microspheres (Miao, Pi et al. 2016), SiO_2/alginate gel composites (Lin, Chen et al. 2008), various nanoparticles (Xu, Huo et al. 2011; Gokhale, Lu et al. 2013; Lima, Araújo et al. 2017; Lin, Liu et al. 2017; Sánchez-Ramírez, Martínez-Hernández et al. 2017; Han, Wang et al. 2018; Simon, Lima et al. 2018), etc. However, vast majority of these preparations is not applicable for natural lignocellulosic substrates conversion due to high price.

Cellulase from *Aspergillus niger* (EC 3.2.1.4.) catalyzes the hydrolysis of endo-1,4-*β*-D-glycosidic linkages in cellulose thus playing crucial role in the pre-fermentation preparation process of different lignocellulosic substrates. Due to widespread availability, low price and less literature data comparing to other LC materials, lignocellulosic fraction of sunflower seed meal was chosen as a substrate. Sunflower seed meal (SSM) is produced in large quantities as a byproduct of oil extraction industry and is currently used as a livestock feed for ruminants and, occasionally, as a fertilizer (Bautista, Parrado et al. 1990). Lignocellulose rich hulls are waste-products of its fractionation process, in which high quality protein concentrate and isolate could be obtained, hence ideally suited for further processing. Hereby, eight carriers with different polarity, porosity and functionality were screened as supports for immobilization of *A. niger* cellulase. Lifetech™ series carriers were chosen due to their good mechanical properties, high chemical resistance and facile separation and regeneration after application. For the most promising support, crucial immobilization parameters – pH, enzyme/support ratio and time were optimized using carboxymethyl cellulose (CMC) as a substrate. Moreover, temperature and pH optima were determined, serving as conditions for application in hydrolysis of lignocellulosic fraction of sunflower seed meal. Obtained preparation was tested in reaction of sunflower seed meal lignocellulosic fraction (SSMLF) hydrolysis under optimized conditions and compared with free enzyme.

MATERIALS AND METHODS

Materials

Cellulase preparation - *Aspergillus niger* cellulase (powder, ≥0.3 units/mg solid) purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany was used as biocatalysts. Substrates were low viscosity carboxymethyl cellulose sodium salt (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and lignocellulosic fraction obtained after fractionation of local sunflower seed meal. Lifetech™ immobilization supports were kind gift of Purolite Corporation (Bala Cynwyd, PA, USA). For DNS reagent preparation following chemicals were used: 3,5-dinitrosalicylic acid (98 %, Acros Organic, New Jersey, USA), NaOH (p.a., Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and potassium sodium tartarate (p.a., Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Salts used for buffer solutions preparation were: Na_2CO_3 (Zorka Pharma, Šabac, Serbia), sodium citrate (Alkaloid, Skopje, Macedonia) NaH_2PO4 (Centrohem, Stara Pazova, Serbia), Na_2HPO_4 (Superlab, Belgrade, Serbia), all analytical grade.

Immobilization procedure

Enzyme immobilization was conducted at room temperature, in 2 ml volume Eppendorf® tubes under stirring on a roller

mixer (Stuart, Paris, France). 25 or 50 mg of immobilization support particles were measured and 0.5 or 1 ml of enzyme solution (predefined concentration of enzyme in immobilization buffer) was added. Within preliminary experiment, for hydrophobic support (ECR8806M) and epoxy/butyl functionalized carrier (ECR8285) immobilization was conducted in 1 M buffer pH 7. Immobilization onto amino functionalized supports was carried out in 50 mM buffer solutions at pH 4.8. After predefined time, supernatant was separated, while particles with immobilized enzyme were washed three times with 0.5 or 1 ml of distilled water and used for activity determination.

Determination of enzyme activity and protein concentration

Activity of free and immobilized cellulase was determined spectrophotometrically by DNS method with 2% CMC in a buffer (50 mM) as a substrate. Predefined amount of free (1 mg/ml) or immobilized (25 or 50 mg in 2 ml) cellulase was incubated with substrate at defined conditions (pH, temperature) and samples were taken during time (up to 5 minutes). 50-125 μl of samples were added to 250 μl of DNS reagent and distilled water to achieve final volume of 0.5 ml and reaction was carried out in a boiling bath for 5 minutes. Then 2 ml of distilled water was added and absorbance was measured at 540 nm. Enzyme activity was determined according to previously described calculations (Ćorović, Mihailović et al. 2017).

Protein concentration was determined using Bradford method, according to the established procedure (Bradford 1976). Bovine serum albumin (BSA) was used as a standard.

Fractionation of sunflower seed meal and hydrolysis of its lignocellulosic fraction

Fractionation of SSM was performed according to standard procedure. By mixing 100 g of minced SSM and 1 l of distilled water, three fractions were obtained – lignoocellulosic fraction (LF, upper layer) was separated by perforated spoon from middle layer (liquid fraction) and precipitate (solid fraction). LF was dried at 180 W of microwave power within 10 cycles lasting 5 minutes, until dry matter content was above 98%.

Hydrolysis reactions were performed at previously optimized conditions in orbitally shaken 100 ml Erlenmeyer flasks in a thermostat. 1 g of substrate was mixed with 10 ml of buffer solution and reaction was initiated by adding predefined amount of free or immobilized enzyme. Samples were taken during reaction and subjected to DNS analysis, according to previously described procedure.

RESULTS AND DISCUSSION

Screening of immobilization supports

A. niger cellulase was initially immobilized onto eight different Lifetech™ carriers with main characteristics shown in Table 1. Displayed performances of obtained preparations are implying that there is significant impact of immobilization support functionality, porosity and polarity on their catalytic activity. Regarding carrier hydrophobicity, it is evident that the more hydrophilic support surface was the higher activity of immobilized cellulase was achieved. Namely, highly hydrophobic Lifetech™ ECR8285 octadecyl acrylate based carrier was the least suitable, followed by two moderately hydrophobic supports comprised of styrene/divinyl benzene with tertiary (Lifetech™ ECR1508) and quaternary (Lifetech™ ECR1604) amino groups. The highest activities were obtained by using four hydrophilic methacrylate carriers with primary amino group. Among them, pronounced influence of pore size (smaller pores 30-60 nm and larger pores 60-120 nm) and functional group spacer arm length (C2 and C6) was observed. It seems that longer spacer arm (C6) provided optimum distance between carrier surface and enzyme molecule, ensuring its unhindered catalytic acting and leading to 37% higher hydrolytic activity. Furthermore, diffusion of substrate molecules was apparently more efficient through the larger pores and facilitated approach to the active sites of cellulase molecules immobilized inside the pores which enabled approximately 30% higher activities comparing to preparations obtained by using carriers with smaller pores.

Table 1.
Screening of immobilization supports.

Lifetech™ support	Type of support	Pore diameter nm	Activity IU/g support
ECR8305F	Amino C2 methacrylate	30-60	13.5
ECR8309F	Amino C2 methacrylate	60-120	19.2
ECR8404F	Amino C6 methacrylate	30-60	21.3
ECR8409F	Amino C6 methacrylate	60-120	30.2
ECR1508	Styrene tertiary amine	n.a.	17.3
ECR1604	Styrene quaternary amine	n.a.	10.0
ECR8806M	Octadecyl methacrylate	40-60	0.0
ECR8285	Epoxy/butyl methacrylate	50-70	3.5

Table 2.
The influence of pH on immobilized cellulase activity.

pH	Bound proteins mg/g support	Activity IU/g support	Specific activity IU/mg bound proteins
3.0	0.12	3.6	30.4
4.0	0.43	18.4	42.3
4.8	0.67	30.2	45.5
6.0	1.07	46.1	43.3
7.0	0.90	11.1	12.3

Therefore, the most promising support for further examination was Lifetech™ ECR8409F so it was applied within subsequent optimization of immobilization conditions and used for the hydrolysis of sunflower seed meal LF.

Investigation of immobilization conditions

Previously established parameters crucial for adsorptive immobilization of enzymes onto solid supports are immobilization buffer pH, initial enzyme concentration and immobilization time. Taking into account that support with primary amino groups was used, it was presumptive that only at appropriate immobilization medium pH values electrostatic interactions between charged amino acid residues from the surface of cellulase molecules and carrier functional groups could be efficiently established. Immobilization was therefore performed at different pH values ranging from 3 to 7 and obtained results are shown in Table 2. As it can be seen, highest expressed activity was accomplished at pH 6. By lowering immobilization pH to 4, gradual activity decrease was observed, while further change to pH 3 led to steep extenuation of activity as well as bound proteins. On the other hand, at pH 7, preparation of very low activity was obtained (11.1 IU/g), although bound protein concentration was only remotely lower in comparison to pH 6 and lowest specific activity (per mg of bound proteins) was achieved, indicating possible partial inactivation of adsorbed enzyme molecules at pH 7. Considering the fact that *A. niger* cellulase has isoelectric point at pH 3.67 it was expected that at pH values below pI repulsive forces between charged amino acid residues are prevailing. Conversely, moving away from pI towards higher pH lead to higher immobilization yields via electrostatic interactions of negatively charged amino acid residues and positively charged primary amino groups of the support and, consequently, to the higher expressed activities. Based on obtained results, further experiments were conducted at pH 6.

Optimal enzyme concentration during immobilization process is strongly dependent on enzyme properties and immobilization support capacity for protein binding. Initial protein concentration was therefore varied in a wide range (1.4-28 mg/g of support) and its influence on several outputs – immobilization yield, immobilized activity and specific activity was monitored. As it is obvious from Fig. 1, within entire range increase of bound proteins per carrier mass was observed, implying that support capacity was not reached. However, only at lower cellulase concentrations proportional increase of expressed immobilized activity was detected.

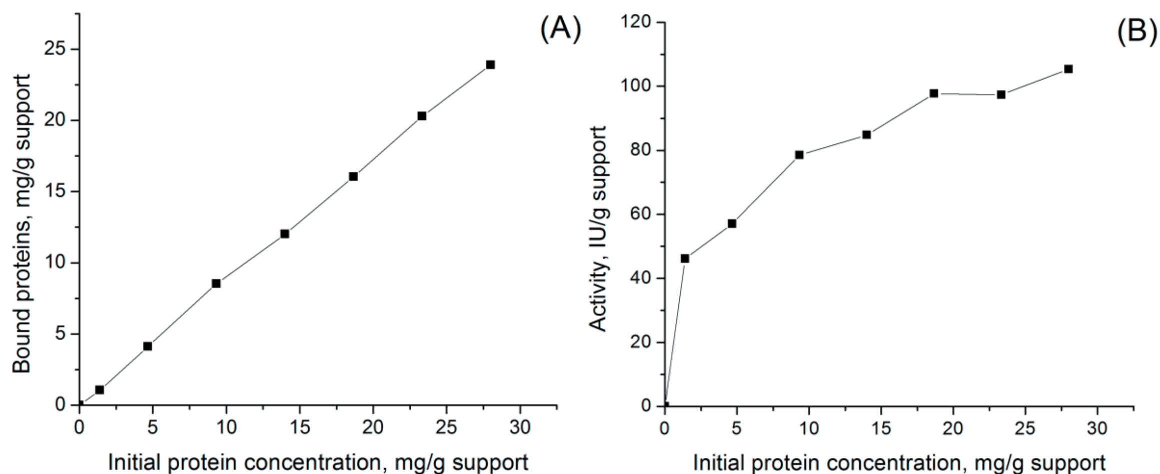

Figure 1. Influence of initial protein concentration on (A) bound proteins and (B) activity of immobilized cellulase

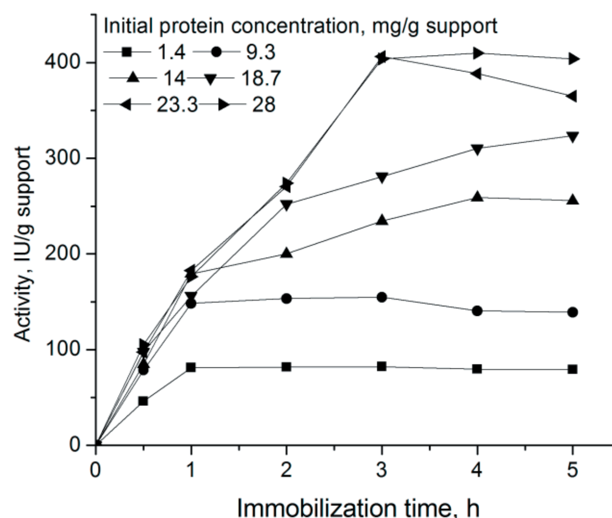

Figure 2. Influence of immobilization time on activity of immobilized cellulase

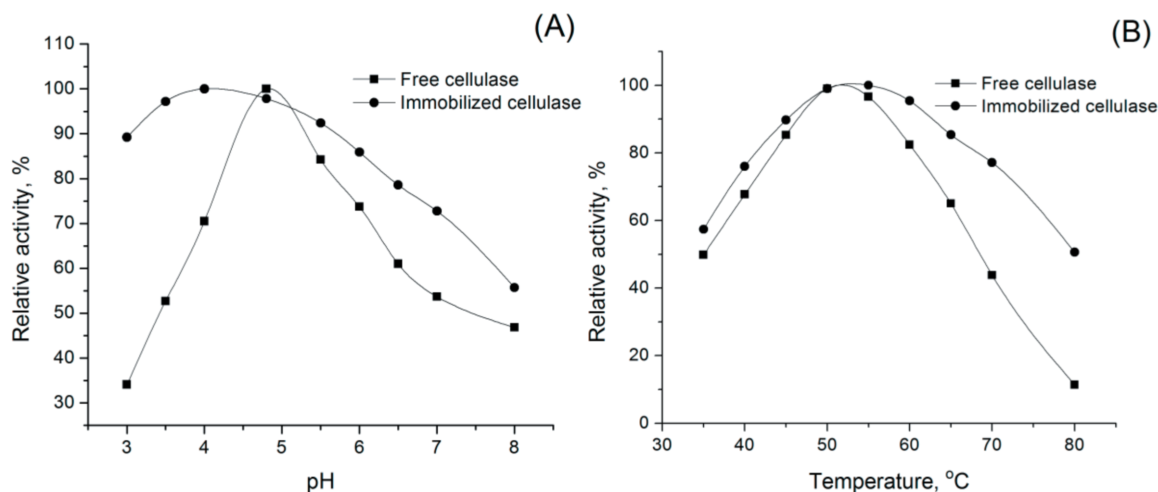

Figure 3. Determination of (A) pH and (B) temperature optimum of free and immobilized cellulase

At higher concentrations, plateau was reached in terms of activity and preparations with 100 IU/g of carrier were obtained. Related immobilization yield was constant within entire examined range - around 80%. For example, when poly(methyl methacrylate) nanoparticles obtained by miniemulsion polymerization method were used for cellulase immobilization, maximum of 59.1% immobilization yield was achieved (Simon, Lima et al. 2018), while by applying SiO_2/alginate gel biocomposites as carriers for cellulase immobilization, 86% enzyme loading was accomplished (Lin, Chen et al. 2008). The most descriptive output regarding catalytic efficacy of enzyme molecules attached to the carrier is specific activity (IU/g of bound proteins) of immobilized enzyme which revealed that, al-though carrier with larger pores was used, there is possibility that catalyst molecules immobilized inside the pores within deeper layers of the carrier particles (usually filled at higher protein concentrations) are more difficult for substrate molecules to access.

Taking into account all these facts, it is not possible to make straightforward conclusion about optimal initial protein concentration, since two opposite demands – obtaining preparation as active as possible at lowest possible costs should be satisfied. Therefore, final examined parameter –immobilization time course was monitored for all tested enzyme concentrations. As presented in Fig. 2, at low initial protein concentrations, immobilization process is rapid (around 1 hour), and within following 23 hours even slight activity loss occurs. On the other hand, at higher initial protein concentrations highest activity of immobilized cellulase was accomplished after 3 hours of immobilization, while prolonged incubation caused severe activity de-crease. Within examined range, maximum achieved activities raised with increase of initial protein content up to 23.3 mg/g of support when preparation with activity of 406 IU/g was obtained.

pH and temperature optimum of immobilized cellulase

Immobilized cellulase obtained under optimized conditions (3 h of immobilization at pH 6 with initial protein concentration 23.3 mg/g support) was characterized in terms of pH and temperature optima, as well as storage, thermal and operational stability. All these features are crucial for efficient application of any immobilized enzyme. Figures 3.A. and 3.B. are showing comparison of relative activity profiles for free and immobilized cellulase at different pH and temperatures. Regarding pH, two simultaneous effects of immobilization were detected – an acidic shift (from pH 4.8 to 4) and broadening of the pH optimum. This feature, which usually requires engineering of the enzyme molecules by replacing amino acid residues that are identified as potentially influencing the pH-activity profile, could be cost-saving for currently favored industrial process which involves exposing the cellulose-rich plant materials to a steam explosion in the presence of strong acid, followed by its neutralization and subsequent digestion with cellulolytic enzymes (Cockburn and Clarke 2011). In that regard, application of catalysts with more acidic pH optimums would be time and cost saving for neutralization step. Furthermore, obtained preparation demonstrated high storage stability accompanied with satisfying temperature optimum – 55 °C. All determined functional characteristics of cellulase immobilized under the most appropriate conditions were promising for its further assessment in a relevant reaction system.

Hydrolysis of sunflower seed meal lignocellulosic fraction

Applicability of immobilized cellulase was examined on a natural substrate – sunflower seed meal LF which could be classified as a by-product of protein concentrate and isolate production process. Prior to hydrolysis, LF was mechanically disrupted into smaller particles by milling. As shown in Figs. 4A and 4B, reaction kinetics was unchanged comparing to free enzyme (when same amount of CMC units was applied), leading to conclusion that no significant influence of diffusion limitations for substrate to access active sites of biocatalyst was present. Unhindered mass transfer is of great importance for utilization of full biocatalytic capacity of immobilized enzymes and could be attributed

to optimal characteristics of immobilization support (highly developed external surface and sufficiently large pore diameter, significant for internal mass transfer). Within examined range of initial cellulase activity, it was observed that increase from 10 to 90 IU/g of SSMLF lead to increased initial hydrolysis rates, where they reached their maximums and stayed unchanged at higher enzyme loadings (Fig. 4B). In that way final yield of approximately 70 mM of glucose equivalents was achieved with 90 U of immobilized cellulase per g of SSMLF, indicating that further increase of biocatalyst concentration is not needeed. Higher reducing sugars concentrations (~200 mM) were previously liberated using sunflower seed meal lignocellulosic fraction (Telli-Okur and Saraçoğlu, 2006). However, acidic treatment based on harsh conditions, namely application of sulphuric acid and high temperatures (90 °C) was applied in that case. Regarding obtained conversions and initial reaction velocities, activity of 90 U per gram of SSMLF could, therefore, be considered as optimal. Interestingly, in a reaction catalyzed by free cellulase, with 90 U/g of SSMLF only 46 mM of glucose equivalents were liberated, while maximum of 70 mM was reached only at doubled enzyme concentration – 180 U/g SSMLF (Fig. 4.A.).

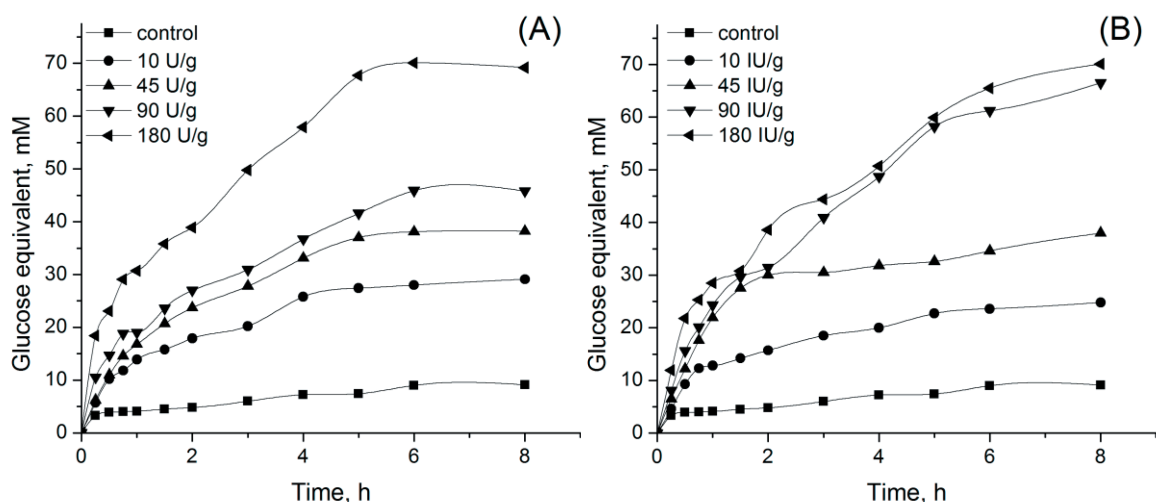

Figure 4. Hydrolysis of sunflower seed meal lignocellulosic fraction by (A) free and (B) immobilized cellulase

CONCLUSIONS

Hereby, possibility for exploitation of catalytic activity of immobilized cellulase from *A. niger* in SSMLF hydrolysis was investigated. Different carriers were screened as immobilization supports and Lifetech^{TM} ECR8409F carrier (amino C6 methacrylate with pore diameter of 60-120 nm) was chosen for further study. At conditions that enabled the highest performance of immobilized enzyme (pH 6, 3 h and initial protein concentration 23.3 mg/g support), preparation with activity of 406 IU/g support was obtained and successfully applied in SSMLF hydrolysis. Preparation demonstrated undiminished catalytic activity comparing to free enzyme and maximum of 70 mM glucose equivalents was produces after 8 h with 90 IU/g of substrate.

ACKNOWLEDGEMENTS

This work was supported by the Serbian Ministry of Education, Science and Technological Development [projects III 46010 and TR 31035].

REFERENCES

1. Bautista, J., Parrado, J., Machado, A. (1990). Composition and fractionation of sunflower meal: Use of the lignocellulosic fraction as substrate in solid-state fermentation. *Biological Wastes, 32*, 225-233.
2. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of

protein-dye binding. *Analytical Biochemistry, 72*, 248-254.

3. Cockburn, D.W., Clarke, A.J. (2011). Modulating the pH-activity profile of cellulase A from Cellulomonas fimi by replacement of surface residues. *Protein Engineering, Design and Selection, 24*, 429-437.
4. Ćorović, M., Mihailović, M., Banjanac, K., Carević, M., Milivojević, A., Milosavić N., Bezbradica D. (2017). Immobilization of *Candida antarctica* lipase B onto Purolite® MN102 and its application in solvent-free and organic media esterification. *Bioprocess and Biosystems Engineering, 40*, 23-34.
5. Gokhale, A.A., Lu, J., Lee, I. (2013). Immobilization of cellulase on magnetoresponsive graphene nano-supports. *Journal of Molecular Catalysis B: Enzymatic, 90*, 76-86.
6. Han, J., Wang, L., Wang, Y., Dong, J., Tang, X., Ni, L. (2018). Preparation and characterization of Fe 3 O 4 -NH 2 @4-arm-PEG-NH 2 , a novel magnetic four-arm polymer-nanoparticle composite for cellulase immobilization. *Biochemical Engineering Journal, 130*, 90-98.
7. Howard, R.L., Abotsi, E., Van Rensburg, E.L. J., Howard, S. (2003). Lignocellulose biotechnology: Issues of bioconversion and enzyme pro-duction. *African Journal of Biotechnology, 2*, 702-733.
8. Ji, H., Pang, H., Zhang, R., Liao, B. (2008). Pretreatment and enzymatic hydrolysis of lignocellulose. *Chemistry Bulletin / Huaxue Tongbao, 71*, 329-335.
9. Jørgensen, H., Kristensen, J.B., Felby, C. (2007). Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities. *Biofuels, Bioproducts and Biorefining, 1*, 119-134.
10. Juturu, V. and J.C. Wu (2014). Microbial cellulases: Engineering, production and applications. *Renewable and Sustainable Energy Reviews, 33*, 188-203.
11. Kuhad, R.C., Gupta, R., Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme Research, 2011*, 10.
12. Kumar, R., Singh, S., Singh, O.V. (2008). Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. *Journal of Industrial Microbiology and Biotechnology, 35*, 377-391.
13. Lima, J.S., Araújo, P.H.H., Sayer, C., Souza, A.A.U., Viegas, A.C., de Oliveira, D. (2017). Cellulase immobilization on magnetic nanoparticles encapsulated in polymer nanospheres. *Bioprocess and Biosystems Engineering, 40*, 511-518.
14. Lin, S., Chen, W., Jiang, N., Fu, F. (2008). SiO_2/alginate gel biocomposites for cellulase immobilization. *Fuhe Cailiao Xuebao/Acta Materiae Compositae Sinica, 25*, 22-27.
15. Lin, Y., Liu, X., Xing, Z., Geng, Y., Wilson, J., Wu, D., Kong, H. (2017). Preparation and characterization of magnetic Fe_3O_4–chitosan nanoparticles for cellulase immobilization. *Cellulose, 24*, 5541-5550.
16. Miao, X., Pi, L., Fang, L., Wu, R., Xiong, C. (2016). Application and characterization of magnetic chitosan microspheres for enhanced immobilization of cellulase. *Biocatalysis and Biotransformation, 34*, 272-282.
17. Sánchez-Ramírez, J., Martínez-Hernández, J. L., Segura-Ceniceros, P., López, G., Saade, H., Medina-Morales, M.A., Ramos-González, R., Aguilar, C.N.,–Ilyina, A. (2017). Cellulases immobilization on chitosan-coated magnetic nanoparticles: application for Agave Atrovirens lignocellulosic biomass hydrolysis. *Bioprocess and Biosystems Engineering, 40*, 9-22.
18. Simon, P., Lima, J.S., Valério, A., De Oliveira, D., Araújo, P.H.H., Sayer, C., De Souza, A.A. U., Guelli U De Souza, S.M.A. (2018). Cellulase immobilization on poly(methyl methacrylate) nanoparticles by miniemulsion polymerization. *Brazilian Journal of Chemical Engineering, 35*, 649-657.
19. Telli-Okur, M., Saraçoğlu, N.E. (2006). Ethanol production from sunflower seed hull hydrolysate by Pichia stipitis under uncontrolled pH conditions in a bioreactor. *Turkish Journal of Engineering and Environmental Sciences*, 30, 317-322.
20. Vaz, R.P., de Souza Moreira, L.R., Ferreira Filho, E.X. (2016). An overview of holocellulose-degrading enzyme immobilization for use in bioethanol production. *Journal of Molecular Catalysis B: Enzymatic, 133*, 127-135.
21. Xu, J., Huo, S., Yuan, Z., Zhang, Y., Xu, H., Guo, Y., Liang, C., Zhuang, X. (2011). Characterization of direct cellulase immo-bilization with superparamagnetic nano-particles. *Biocatalysis and Biotransformation, 29*, 71-76.

ИМОБИЛИЗАЦИЈА *ASPERGILLUS NIGER* ЦЕЛУЛАЗЕ НА LIFETECH™ НОСАЧЕ И ПРИМЕНА У РЕАКЦИЈИ ХИДРОЛИЗЕ ЛИГНОЦЕЛУЛОЗНЕ ФРАКЦИЈЕ СУНЦОКРЕТОВЕ САЧМЕ

Марија М. Ћоровић*[1], Милица Б. Симовић[1], Ана Д. Миливојевић[2], Катарина М. Бањанац[2], Катарина Д. Катић[3], Дејан И. Безбрадица[1]

[1] Универзитет у Београду, Технолошко-метлуршки факултет, Карнегијева 4, 11000 Београд, Србија
[2] Универзитет у Београду, Технолошко-метлуршки факултет, Иновациони центар, Карнегијева 4, 11000 Београд, Србија
[3] Институт за хигијену и технологију меса, Каћанског 13, 11000 Београд, Србија

Сажетак: Целулазе су ензими који катализују хидролизу целулозе и у широкој су примени у бројним гранама индустрије. У последње време, могућност њихове примене у третирању различитих опадних агроиндустријских сировина у циљу добијања биогорива интензивно се истражује. Да би се повећале стабилност и економичност њихове примене, потребно је уместо слободног ензима користити имобилисане форме. У овом истраживању осам Lifetech™ носача различитих поларности, порозности и функционалних група. тестирано је за имобилизацију целулазе продуцента *Aspergillus niger*. Најпогоднији носач био је на бази метакрилата, са примарним амино групама, C6 „дугом ножицом" и порама пречника 60-120 nm - Lifetech™ ECR8409F. За овај носач одређени су најзначајнији услови имобилизације и након 3 сата на pH 6, при почетној концентрацији протеина од 23,3 mg/g носача добијена је имобилисана целулаза активности 406 IU/g (са карбокси метил целулозом као супстратом). Овај препарат успешно је примењен у реакцији хидролизе лигноцелулозне фракције сунцокретове сачме, која преставља нуспроизвод фракционисања сунцокретове сачме при добијању фракција богатих протеинима. Почетне брзине реакције и приноси редукујућих шећера били су непромењени у односу на слободан ензим, указујући да није било значајног утицаја дифузионих лимитација при приласку супстрата активним центрима молекула *A. niger* целулазе имобилисане на Lifetech™ ECR8409F носач.

Кључне речи: *β-глуканаза, имобилизација, сунцокретова сачма, лигноцелулоза, сахарифи-кација*

Received: 19 August 2019

Received in revised form: 10 September 2019

Accepted: 30 September 2019