Zavod za zaštitu prirode Crne Gore

Ministarstvo održivog razvoja i turizma

NAUČNI SKUP SA MEĐUNARODNIM UČEŠĆEM ▌INTERNATIONAL CONFERENCE

ZAŠTITA PRIRODE U XXI VIJEKU

NATURE PROTECTION IN XXIST CENTURY

ZBORNIK REFERATA, REZIMEA REFERATA I POSTER PREZENTACIJA (KNJIGA BR 1)

Radovi su objavljeni u obliku dobijenom od autora

▌

PROCEEDINGS OF THE CONFERENCE (BOOK No 1)

Articles published in the Proceedings are given with the content received from the authors

20 – 23 Septembar 2011.
Žabljak, Crna Gora / Montenegro

GENERALNI POKROVITELJ
Ministarstvo održivog razvoja i turizma, Vlada Crne Gore
Ministar Predrag Sekulić

ORGANIZATOR
Zavod za zaštitu prirode Crne Gore

Kompjuterski slog
Milica Jovanović, Zavod za zaštitu prirode Crne Gore

ŠTAMPA
AP Print, Podgorica

TIRAŽ
250 komada

ORGANIZACIONI ODBOR

PREDSJEDNIK
Dr Zlatko Bulić, direktor Zavoda za zaštitu prirode Crne Gore

ČLANOVI:
Mr Ivana Vojinović, pomoćnik Ministra održivog razvoja i turizma
Dr Aleksandar Joksimović, direktor Instituta za biologiju mora
Mr Luka Mitrović, direktor Hidrometeorološkog zavoda Crne Gore
Daliborka Pejović, direktorica Agencije za zaštitu životne sredine
Rade Gregović, direktor JP „Nacionalni parkovi Crne Gore“
Branko Žugić, direktor Geološkog zavoda Crne Gore
Rajko Barović, direktor JP “Morsko dobro“
Nada Medenica, direktor JU“CETI“,Podgorica
Prof. dr Vladimir Pešić, Šef odsjeka za biologiju – PMF, Podgorica
Ondrej Vizi, direktor Prirodnjačkog muzeja Crne Gore
Zoran Janković, direktor Uprave za vode
Tomo Pajović, direktor NP „Durmitor“
Mr Darko Brajušković, direktor NP „Biogradska gora“
Mr Zoran Mrdak, direktor NP „Skadarsko jezero“
Baćko Uskoković, direktor NP „Lovćen“
Dr Dragan Roganović, Zavod za zaštitu prirode Crne Gore
Mr Vasilije Bušković, Zavod za zaštitu prirode Crne Gore
Dr Vesna Mačić, Institut za biologiju mora Kotor
Dr Ruža Ćirović, Zavod za zaštitu prirode Crne Gore
Mr Sead Hadžiablahović, Zavod za zaštitu prirode Crne Gore
Dr Goran Barović, Filozofski fakultet Nikšić – Odsjek za Geografiju
Dr Gojko Nikolić, Filozofski fakultet Nikšić – Odsjek za Geografiju
Mr Gordana Kasom, Zavod za zaštitu prirode Crne Gore
Milenko Damjanović, direktor Centra za savremenu umjetnost Crne Gore
Miroje Džabasan, direktor Centra za kulturu, Žabljak

POČASNI ODBOR

ČLANOVI:
Prof. dr Predrag Miranović, rektor UCG
AkademikPerko Vukotić,, CANU
Akademik Božidar Nikolić, DANU
Akademik Milorad Mijušković, CANU
Akademik Branko Radojičić, DANU
Akademik Ljubomir Berberović, ANUBiH, Sarajevo i CANU Podgorica
Akademik Midhat Ušćuplić, ANUBIH
Slavoljub Stijepović, Ministar prosvjete i sporta
Dr Sonja Vlahović, Ministar nauke
Tarzan Milošević, Ministar poljoprivrede i ruralnog razvoja,
Branislav Mićunović, Ministar kulture i medija,
Branimir Gvozdenović, Podgorica,
Predrag Nenezić, Podgorica
Dr Miodrag Gomilanović, Podgorica
Predrag Jelušić, direktor HTP „Primorje“ Tivat
Prof. dr Blagoje Cerović, dekan, Filozofski fakultet, Nikšić
Prof. dr Radovan Bakić, Filozofski fakultet, Nikšić
Dr Dušan Dragović, naučni savjetnik, Podgorica
Dr Vasilije Radulović, naučni savjetnik
Prof. dr Mihailo Burić, Filozofski fakultet Nikšić
Prof. dr Slobodan Kasalica, Filozofski fakultet, Nikšić
Ana Mišurović, JU „CETI“, Podgorica
Dr Đorđije Drecun, Podgorica
Mr Salija Adrović, srednjo-školski centar Berane
Zoran Tomić, sekretar Ministarstva održivog razvoja i turizma
Daniel Vincek, Botanička bašta planinske flore Crne Gore, Kolašin
Isailo Šljivančanin, predsjednik opštine Žabljak

NAUČNI ODBOR

PREDSJEDNIK
Akademik Vukić Pulević

ČLANOVI:
Akademik Gordan Karaman – CANU. Podgorica
Akademik Vladimir Stevanović, SANU - Beograd
Akademik Sulejman Redžić, ANUBiH - Sarajevo
Akademik Vlado Matevski, PMF - Skoplje
Prof. dr Sreten Mandić, Institut za biologiju mora Kotor
Prof. dr Miljan Radović, Beograd
Prof. dr Milutin Lješević, Filozofski fakultet, Nikšić
Prof. dr Ivica Radović, Beograd
Dr Deni Porej, WWF
Dr Boris Erg, IUCN
Davorin Marković, Državni zavod za zaštitu prirode Hrvatske, Zagreb
Prof. dr Jasenka Topić, PMF, Zagreb
Prof. dr Nenad Stavretović, Zavod za zaštitu prirode Srbije
Prof. dr Ljupče Melovski, PMF - Skoplje
Dr Darij Krajičić, Zavod za zaštitu prirode Slovenije
Dr Boštjan Surina, Prirodoslovni muzej, Rijeka
Dr Biljana Panjković, Zavod za zaštitu prirode Vojvodine
Prof. dr Branislav Glavatović, Seizmološki zavod Crne Gore
Prof. dr Predrag Stanišić, PMF, Univerzitet Crne Gore
Prof. dr Rifat Škrijelj, PMF-Odsjek za biologiju Sarajevo
Dr Zlatko Bulić, Zavod za zaštitu prirode Crne Gore
Prof. dr Mićko Radulović, Građevinski fakultet Univ. Crne Gore
Prof. dr Milorad Gojković, Institut za biologiju mora Kotor
Prof. dr Zoran Kljajić, Institut za biologiju mora, Kotor
Prof. dr Nenad Vuksanović, Institut za biologiju mora, Kotor
Prof. dr Staniša Ivanović, Filozofski fakultet, Nikšić
Prof. dr Miroslav Doderović, Filozofski fakultet, Nikšić
Mr Dragutin Nedić, Podgorica
Mr Halil Markišić, Rožaje
Prof. dr Milivoje Purić, PMF – Odjek za biologiju UCG
Prof. dr Drago Marić, PMF – Odjek za biologiju UCG
Prof. dr Dmitar Lakušić, PMF – Odsjek za biologiju, Beograd
Prof.dr Vladimir Ranđelović, PMF, Niš
Prof. dr Marash Rakaj, PMF Skadar, Albanija
Dr Slobodan Vukčević, Podgoria
Prof. dr Natalija Perović, Biotehnički fakultet, Podgorica

SEKRETARIJAT

ČLANOVI:
Mr Nataša Stanišić, Zavod za zaštitu prirode Crne Gore
Mr Milka Rajković, Zavod za zaštitu prirode Crne Gore
Vesna Jovović, Zavod za zaštitu prirode Crne Gore
Mr Rajko Tripić, Zavod za zaštitu prirode Crne Gore
Mr Sonja Kralj, Zavod za zaštitu prirode Crne Gore
Jelena Nikčević, Zavod za zaštitu prirode Crne Gore
Darko Saveljić, Zavod za zaštitu prirode Crne Gore
Mr Biljana Telebak, Zavod za zaštitu prirode Crne Gore
Nataša Miličković, Zavod za zaštitu prirode Crne Gore
Milica Jovanović, Zavod za zaštitu prirode Crne Gore
Mitar Baranin, direktor hotela „Planinka“, Žabljak

GENERALNI SEKRETAR:
Mr Vasilije Bušković

IMMOBILIZATION OF HORSE RADISH PEROXIDASE ON DIFFERENT MACROPOROUS GLYCYDIL METHACRYLATES FOR WASTEWATER TREATMENT

OLIVERA PRODANOVIĆ[1], MILOŠ PROKOPIJEVIĆ[1], DRAGICA SPASOJEVIĆ[1], RADIVOJE PRODANOVIĆ[2], ŽELJKO STOJANOVIĆ[3], KSENIJA RADOTIĆ HADŽI-MANIĆ[1]

Abstract

Removal of phenolic compounds from wastewaters has been investigated by many researchers and it has been shown that horse radish peroxidase can react with aqueous phenolic compound in the presence of hydrogen peroxide and form non-soluble materials that could be easily removed from the aqueous phase. Main disadvantage of this process is enzyme inactivation. In order to increase stability of enzyme and yield of biocatalytic reaction peroxidases were immobilized on different materials. The aim of this study was to test different macroporous glycidyl methacrylates as carriers for enzyme immobilization.
Immobilization was performed using carriers with different pore sizes (40, 50, 120 and 200 nm) and glutaraldehyde as activation method. All immobilized enzymes were characterized using pyrogallol and H_2O_2 as substrates (13 mM and 10 mM, respectively), and specific activity and immobilization yield were determined.
Our data demonstrate that the best carrier for immobilization of horse radish peroxidase was SGE EDA 20/12 with pore size of 120 nm.
Key words: horse radish peroxidase, glycidyl methacrylate, immobilization, pyrogallol, glutaraldehyde.

Introduction

High concentration of pollutants generated through various industrial activities like manufacturing of resins, plastics, textiles, pulp and paper, coal conversion, etc. are contaminating aquatic environment. Among them, phenolic compounds like phenol and chlorogenic derivatives are one of the major pollutants of industrial wastewaters. Hence phenolic compounds removal is of great importance NEJATI & ALEMZADEH (2009).
In recent years enzyme catalyzed polymerization and precipitation process has been explored as a new method for treatment of phenol solutions. Among these methods, various peroxidases are used as polymerization agents like horse-radish peroxidase, soybean peroxidase, tomato peroxidase etc. HUSAIN (2010).
However due to the high cost of the enzyme and instability in the presence of peroxide and side reactions much effort has been devoted to stabilization of peroxidases by adding additives like PEG and to reuse enzyme by immobilization ARUCA & BAYRAMOGLU (2008).
Macroporous copolymer of ethylene glycol dimethacrylate and glycidyl methacrylate, poly(GMA-co-EGDMA) has been previously used for immobilization of enzymes PRODANOVIC et al (2006). It has a large surface area, hydrophilic microenviroment, a number of active epoxide groups that could be easily modified to amino groups and an ability for obtaining targeted porous structure JOVANOVIC et al (1996). These variety of porous structures that could be obtained offer a possibility to find a copolymer with suitable surface characteristics for high activity of investigated enzyme.
In this work we tested different macroporous glycidyl methacrylates for HRP immobilization by glutaraldehyde and optimized immobilization conditions.

Materials and methods

[1] Institute for Multidisciplinary Research, University of Belgrade, Kneza Visešlava 1, 11030 Belgrade, Serbia, oliverap@imsi.rs; oprodanovic@gmail.com
[2] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia, rprodano@chem.bg.ac.rs
[3] Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11001 Belgrade, Serbia,

Material

Horseradish Peroxidase, glutaraldehyde and pyrogallol were from Sigma-Aldrich. Macroporous glycidyl methacrylate with different pore sizes was obtained by suspension polymerization as previously described by JOVANOVIĆ et al. (1996). Polymer was modified my ethylene diamine as was previously described PRODANOVIC et al (2006).

Immobilization

0.50 g of the dry copolymer was rinsed and dezaerated in water, activated with 2.5 % glutaraldehide in 50 mM sodium phosphate buffer pH 7.0 for 2 hours. After that the copolymer was rinsed with water and incubated with different amounts of horse radish peroxidase (HRP) in sodium phosphate buffer pH 7.0 over the night at 4 ^{o}C. The immobilized enzyme was then rinsed several times with 1 M NaCl in 50 mM sodium phosphate buffer pH 7.0 (washings are combined and stored for activity measurements) and stored at 4 oC in 50 mM sodium phosphate buffer pH 7.0 till use.

Enzyme Activity

Peroxidase activity was measured in 3 mL of 13 mM pyrogallol in 0.1 M sodium phosphate buffer pH 7.0 using 3-10 mg of immobilized enzyme. Reaction was started by adding 30 μl 0.97 M hydrogen peroxide. During first 3 minutes, reaction was monitored spectrophotometrically at 420 nm. 1 IU of enzymatic activity was amount of enzyme that can oxidize 1 μmol of pyrogallol in 1 min at 25°C and pH 7.

Results and Discussion

In order to have immobilized enzyme with high activity and stabillity it is necessary to use suitable carrier and to optimize immobilization conditions like amount of added enzyme and time of immobilization. That's why we used macroporous glycidyl methacrylate with different macroporous structure and pore characteristics (Table 1).

Table 1: Surface characteristics of glycidyl methacrylate.

	Pore size
MP3 150-300	40 nm
EDA 10/12	50 nm
EDA 20/12	120 nm
SGE 15/16	200 nm

In order to optimize amount of enzyme used in immobilization, we applied 1, 5, 25 and 50 mg per 1 g of dried polymer and measured specific activity of obtained immobilized HRP (Table 2).

Table 2: Dependence of specific activity of the immobilized HRP [IU/g] on the amount of added enzyme (mg) per gram of dry polymer and type of used polymer.

	1 mg/g	5 mg/g	25 mg/g	50 mg/g
EDA 20/12	2.92	8.59	23.6	25.9
EDA 10/12	2.75	7.86	11.6	14.3
SGE 15/16	2.39	8.11	16.0	27.6
MP3 150-300	0.12	1.41	6.33	10.8

From obtained results it could be seen that type of the used polymer was very important for activity of immobilized enzyme. This could be explained by different pore structure of used polymers that was influencing diffusion of substrate and product through polymer beads. Also amount of applied enzyme was critical for succesfull immobilization. By applying more enzyme, higher activity was usually obtained. At the end highest specific activity (25.9 IU/g) of

immobilized HRP was obtained using batch of polymer named EDA 20/12 with pore size of 120 nm and by applying 50 mg of enzyme per 1 g of dry polymer.
In order to check if activity of immobilized enzyme can be further increased we tried to optimized incubation time used in HRP immobilization by increasing it more than 2 h (Table 3).

Table 3: Dependence of specific activity of the immobilized HRP [IU/g] on the incubation time during immobilization.

t (h)	2	6	24	48
EDA 10/12	15.1	16.9	20.1	32.0

By increasing the time of incubation from 2 h that is usually used during glutaraldehyde immobilization, we managed to double specific activity of immobilized enzyme after 48 h of incubation.

Conclusion

We tested different macroporous glycidyl methacrylates for HRP glutaraldehyde immobilization and optimized immobilization conditions with respect to enzyme amount and time of incubation. Highest activity of immobilized enzyme was obtained using polymer named EDA 20/12 and 50 mg of enzyme per 1 g of dry polymer. By further increasing time of incubation specific activity could be doubled. High activity of immobilized enzyme that was obtained is very important for succesful long term use of obtained immobilized HRP in continuous phenol removal from wastewater.

Acknowledgment: This research was supported in grant No. 173017 by Ministry of Science and Education of Republic of Serbia.

References

1. NEJATI, S., ALEMZADEH, I., 2009: Phenols removal by immobilized horseradish peroxidase. Journal of Hazardous Materials, V.166, p. 1082-1086.
2. HUSAIN, Q., 2010: Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: review. Review of Environmental Science and Biotechnology, V.9, p. 117-140.
3. ARICA, M.Y., BAYRAMOGLU, G., 2008: Enzymatic removal of phenol and p-chlorophenol in enzyme reactor: Horseradish peroxidase immobilized on magnetic beads. Journal of Hazardous Materials, V.156, p. 148-155.
4. PRODANOVIĆ, R., MILOSAVIĆ, N., JOVANOVIĆ, S., PRODANOVIĆ, O., ĆIRKOVIĆ VELIČKOVIĆ, T., VUJČIĆ, Z., JANKOV, R.M., 2006: Activity and stability of soluble and immobilized α-glucosidase from baker's yeast in cosolvent systems. Biocatalysis and Biotransformation, V. 24, p. 195-200.
5. JOVANOVIĆ, S.M., NASTASOVIĆ, A., JOVANOVIĆ, N.N., JEREMIĆ, K., 1996: Targeted porous structure of macroporous copolymers based on glycidyl methacrylate. Mater. Sci. Forum V. 214, p. 155-162.