Procesing '22

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Holiday Inn, Beograd

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pisanih za 35. Međunarodni kongres o procesnoj industriji
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Društvo za procesnu tehniku
Kneza Miloša 7a/II,
11000 Beograd

Predsednik Društva za procesnu tehniku
pri SMEITS-u
prof. dr Aleksandar Jovović, dipl. inž.

Urednici
Prof. dr Dušan Todorović, dipl. inž.
Prof. dr Miroslav Stanojević, dipl. inž.
Prof. dr Aleksandar Jovović, dipl. inž.

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Savez mašinskih i elektrotehničkih
inženjera i tehničara Srbije (SMEITS),
Društvo za procesnu tehniku
Kneza Miloša 7a/II, 11000 Beograd
Tel. +381 (0) 11 3230-041, +381 (0) 11 3031-696,
tel./faks +381 (0) 11 3231-372
E-mail: office@smeits.rs
web: www.smeits.rs

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Pomoć u organizovanju Procesinga '22 dali su članovi Katedre za procesnu tehniku Mašinskog fakulteta Univerziteta u Beogradu i mnogih drugih fakulteta iz Srbije.

Ovogodišnji skup završava se posetom novom Centru za upravljanje otpadom u Vinči.

U Beogradu
juni 2022.

SADRŽAJ

Procesne tehnologije

Projektovanje, izgradnja, eksploatacija i održavanje procesnih postrojenja

Inženjerstvo životne sredine i održivi razvoj u procesnoj industriji

Energetska efikasnost u procesnoj industriji

Procesi i postrojenja u pripremi i prečišćavanju vode u procesnoj industriji

Modelovanje i optimizacija procesnih i termoenergetskih postrojenja

Merenja i upravljanje u procesnoj industriji

Menadžment kvaliteta i standardizacija u organizacijama

Oglasni deo

IMOBILIZACIJA PEROKSIDAZE IZ KROMPIROVIH LJUSKI U OBLIKU UMREŽENIH ENZIMSKIH AGREGATA ZA "ZELENU" RAZGRADNJU ANTRAHINONSKE BOJE

TO BE FREE OR NOT TO BE FREE: CROSS-LINKING OF POTATO PEEL PEROXIDASE FOR "GREEN" DEGRADATION OF ANTHRAQUINONE DYE

Milica SVETOZAREVIĆ[1*], Nataša ŠEKULJICA[1], Ana DAJIĆ[1], Marina MIHAJLOVIĆ[1], Zorica KNEŽEVIĆ-JUGOVIĆ[2], Dušan MIJIN[2]

[1] Innovation Center, Faculty of Technology and Metallurgy in Belgrade, Belgrade

[2] University of Belgrade, Faculty of Technology and Metallurgy, Belgrade

Boje koje se koriste u tekstilnoj industriji predstavljaju opasnost po životnu sredinu zbog toga što veći deo na kraju završi u vodotokovima. Kako konvencionalne tretmane karakteriše potreba za velikim količinama reagenasa, visoki troškovi procesa kao i pojava značajnih količina otpadnih materija na kraju procesa, stalno se radi na pronalasku novih efikasnijih tretmana otpadne vode. Za razliku od klasičnih tretmana, primena enzima, kao ekološki benignih biokatalizatora, predstavljaju „zeleno" rešenje ovog problema. Kako bi se smanjili troškovi proizvodnje enzima predložena je njihova izolacija iz otpadnih materijala. Dugotrajni, komplikovani postupci prečišćavanja otpadnih materijala mogli bi se zaobići izolacijom u obliku sirovog ekstrakta. Imobilizacija enzima omogućila bi njihovu višekratnu upotrebu što bi doprinelo efikasnosti procesa. U izvedenim eksperimentima, ispitan je uticaj taložnog reagensa i koncentracije sredstva za umrežavanje na aktivnost enzima i efikasnost imobilizacije. Nakon umrežavanja, enzimski agregati su su korišćeni za razgradnju boje Acid Violet 109 i optimizovani su sledeći parametri: pH, koncentracija enzima, koncentracija vodonik-peroksida i koncentracija boje. Tokom eksperimenata ispitana je i operativna stabilnost umreženih agregartata.

Ključne reči: *peroksidaza; čistija proizvodnja; poljoprivredni otpad; antrahinonska boja*

Enzyme immobilization is a convenient technique for reuse of enzymes – it is one of the advantages that contributes for enhanced productivity and efficacy of these processes. Enzyme stability and their recovery from a reaction mixture are just a couple of the many benefits that can be acquired by immobilization [1]. These features give opportunity for enzymatic application at industrial scale. The enzyme used in this study is peroxidase, an oxidoreductase that oxidizes variety of organic pollutants such as: phenols, textile dyes and pharmaceutically active compounds [2]. The use of potato peel as a source of peroxidase for degradation of textile dye contributes to the sustainability of the treatment. The simple isolation of peroxidase as a crude extract adds up to the cost effectiveness of the product (peroxidase). Given the fact that peroxidase is an eco-friendly biocatalyst, an effort is made to find a suitable 'green' way of immobilization. Glutaraldehyde is the most common cross-linker used for the same kind of immobilization [3]. However, it is a toxic compound, so it is replaced by alternative compounds such as natural polysaccharides. One of them is pectin that can be oxidized in order to introduce aldehyde groups, which in turn react with the amino-groups from the amino-acid residues and form Schiff bases [4], [5]. Afterwards, the cross-linked peroxidase can be applied for oxidation reaction.

In this study, peroxidase was isolated from waste material – potato peel as a crude extract. The enzymatic crude extract was precipitated with different reagents, in order to find the most suitable one. Next, the influence of the cross-linker concentration was examined. The cross-linked potato peel peroxidase was used for biodegradation of the anthraquinone dye Acid Violet 109. The process parameters: pH, reaction time, enzyme, hydrogen peroxide and dye concentration were optimized for achieving the maximal degradation rate. The operational stability, as a key parameter in immobilized systems was also examined.

Key words: *peroxidase; cleaner production; agroindustrial waste; anthraquinone dye*

[*] Corresponding author, e-mail: msvetozarevic@tmf.bg.ac.rs

1 Materials and Methods

1.1 Materials

Potato peels were obtained from the local groceries; C.I. Acid Violet 109 from DCC colorants (Ningbo,); pyrogallol, monobasic potassium phosphate anhydrous, dibasic potassium phosphate trihydrate, hydrogen peroxide 35% from Carlo Erba; sodium acetate from Alkaloid; and glacial acetic acid from Lach:ner. Apple pectin was purchased from Sigma-Aldrich. All the reagents were of analytical grade.

1.2 Influence of the precipitating reagent on the enzyme cross-linking

The influence of the precipitating reagent was examined using ethanol, 2-propanol, acetone and ammonium sulfate. The precipitation with organic solvents was done by mixing the crude enzyme extract with cold solvent in ratio 1:3. Precipitation with ammonium-sulfate was done by making 90% saturated solution. The mixtures were incubated for 1h at 4°C, and afterwards centrifuged for 10 min at 4°C and 10000 RPM. The precipitate was suspended in 0.5 mL acetic buffer (pH 4.0, 50 mM). The enzyme activity was measured by using pyrogallol as a standard substrate, and the protein content was determined by the modified Lowry method. The activity recovery was calculated by the following equation:

$$activity\ recovery = \frac{activity\ of\ crosslinked\ peroxidase\ (IU)}{activitiy\ of\ free\ peroxidase\ used\ for\ crosslinking\ (IU)} \quad (1)$$

1.3 Influence of the cross-linker concentration on the enzyme immobilization

The influence of the cross-linker concentration on the enzyme immobilization was examined by varying the oxidized pectin concentration in the range 0.1-2% (w/v). After the addition of ammonium-sulfate, the reaction mixture was incubated 1h at 4°C. Next, oxidized pectin was added to the mixture, which was left overnight at 4°C. The enzyme aggregates were separated from the supernatant by centrifugation: 10 min at 4°C and 13000 RPM. The precipitate was washed by 50 mM acetic buffer (pH 4.0) and subjected to activity determination. The supernatant was subjected to activity and protein content measurements.

1.4 Optimization of pH and reaction time for degradation of Acid Violet 109 by cross-linked potato peel peroxidase

The optimization of pH was performed by varying the pH in the range 3 – 9. The dye (30 mg/L) was dissolved in buffer solutions, where the reaction volume was 50 mL. The biodegradation rate was monitored spectrophotometrically at λ = 590 nm. When the change in absorbance became insignificant, it was considered that an equilibrium was achieved, and the reaction time was adopted. The biodegradation rate was calculated by the folowing equation:

$$Biodegradation\ rate\ (\%) = \frac{A_0 - A_t}{A_t} \cdot 100 \quad (2)$$

Where A_0 is the absorbance of the dyed solution at the beginning, while A_t is the absorbance of the dyed solution at a given time.

1.5 Optimization of enzyme, hydrogen peroxide and dye concentration for degradation of Acid Violet 109 by cross-linked potato peel peroxidase

The enzyme concentration was varied in the range of 0.1 – 1 IU. A 30 mg/L dye was dissolved in a citrate buffer (pH 3.0) in a total volume of 50 mL. After the addition of the cross-linked peroxidase and 0.1 mM hydrogen peroxide, the reaction started and was monitored for 70 min, spectrophotometrically at λ=590 nm. For hydrogen peroxide concentration optimization, H_2O_2 concentration was varied in the range 0,1 – 1 mM. First, the dye was prepared in citric buffer (pH 3.0) with a total

volume of 50 mL. Next, 0.8 IU cross-linked potato peroxidase was added. After the addition of H_2O_2, the reaction was monitored for 70 min. For determination of optimal dye concentration for Acid Violet biodegradation, the concentration of the dye was varied 10 – 60 mg/L. The reaction was performed at room temperature and constant mixing 200 RPM.

1.6 Operational stability of cross-linked potato peel peroxidase for degradation of Acid Violet 109

For determination of the operational stability of cross-linked potato peroxidase, the biodegradation reaction of Acid Violet 109 is monitored in a batch system. After every biodegradation cycle, the cross-linked enzyme was separated by vacuum filtration, washed with 50 mM acetic buffer pH 4.0 and added to a new cycle of biodegradation. The steps were repeated until there was significant change in the biodegradation rate.

2 Results and Discussion

2.1 Influence of the precipitating reagent on the enzyme cross-linking

The influence of the precipitating reagent on the potato peel peroxidase immobilization was examined by precipitating the enzyme by 2-propanol, ethanol, acetone and ammonium sulfate. The results are shown in Figure 1. It can be concluded that ammonium sulfate gave the best results, with an activity recovery of 58.32 ± 1.61 %. Similar results were obtained by Rehman et al. [6]. Ethanol and 2-propanol showed the least activity recovery: 23.73 ± 1.66 i 23.29 ± 1.63 %, respectively. The specific activity had the highest value when ammonium sulfate was used as a pecipitating agent - 1.24 ± 0.09 IU/mg.

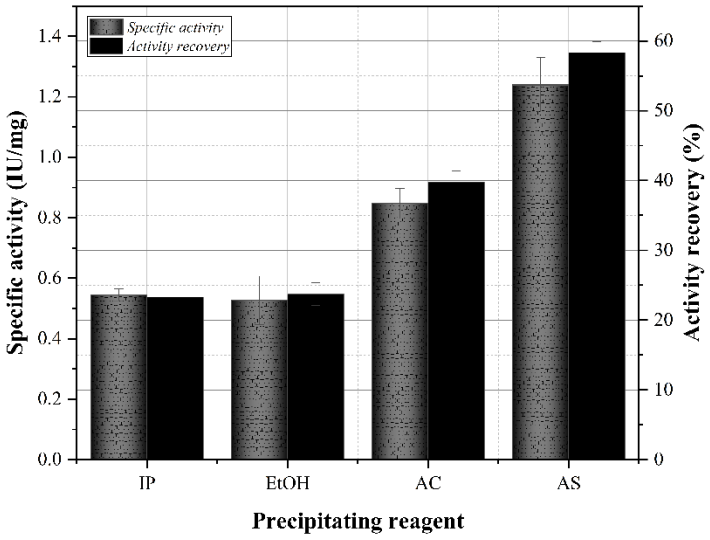

Figure 1. Influence of the precipitation reagent on the peroxidase specific activity and activity recovery (IP – 2-propanol, EtOH – ethanol, AC – acetone, AS – ammonium sulphate)

2.2 Influence of the cross-linker concentration on the enzyme immobilization

The concentration of the oxidized pectin was varied in the range 0.1 – 2 %. The immobilization was performed for 24h, at 4°C with constant stirring. The results are shown on Figure 2. From Figure 2, the optimal pectin concentration is 0.1%, where 0.735 ± 0.08 IU/mg specific activity was achieved. With increase of pectin concentration above 0.1 %, the specific activity decresased. Kumar et al. came across the same conclusion when they made an effort of cross-linking laccase by glutaraldehyde. Lower cross-linker concentration lead to a better enzyme activity.

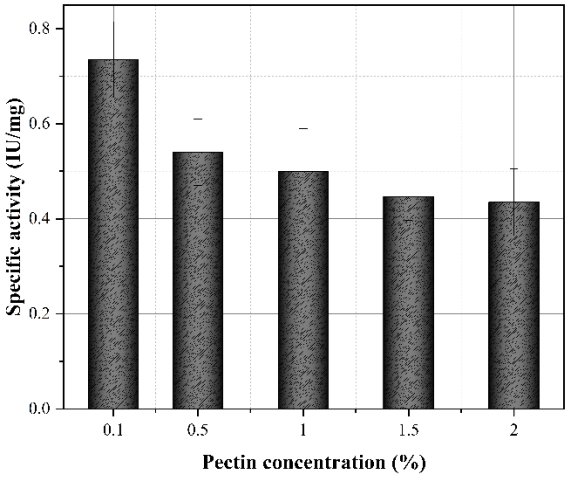

Figure 2. Influence of the cross-linker concentration on the enzyme specific activity

2.3 Optimization of pH and reaction time for degradation of Acid Violet 109 by cross-linked potato peel peroxidase

In order to determine the optimal pH value at which the Acid Violet 109 biodegradation rate is maximal, the pH of the reaction mixture is varied in the range of 2 – 6. The results are shown in Fig. 3a. From the Figure, it can be seen that the optimal pH value is 3. In earlier studies, where free potato peel peroxidase is used, the optimal pH value was 4 [7]. After 70 min of biodegradation, the change in the biodegradation rate was insignificant, so this was adopted as a reaction time.

2.4 Optimization of enzyme, hydrogen peroxide and dye concentration for degradation of Acid Violet 109 by cross-linked potato peel peroxidase

The enzyme concentration was varied in the range 0.1 – 1 IU. As it can be seen from Figure 3b, the biodegradation rate increased as the enzyme concentration was increased. The biodegradation rate was 34.17 ± 1.52 % i 34.24 ± 1.48 % when 0.8 and 1 IU was used, respectively. Enzyme activity of 0.8 IU was used for further experiments. The concentration of hydrogen peroxide was examined in the range of 0.1 – 1 mM (Fig. 3c). The highest biodegradation rate of 52.86 ± 1.56 % was achieved with 0.4 mM H_2O_2. Further increase in hydrogen peroxide concentration had an inhibitory effect on the reaction rate. The dye concentration was varied in the range 10 – 100 mg/L. From Fig. 3d can be concluded that optimal dye concentration was 10 mg/L with 82.2 ± 1.65 %. Bilal et al. have also concluded from their research that immobilized horseradish peroxidase shows better performance at low dye concentrations [8]. The increase of dye concentration lead to a decrease in the biodegradation of AV109 by potato peel peroxidase.

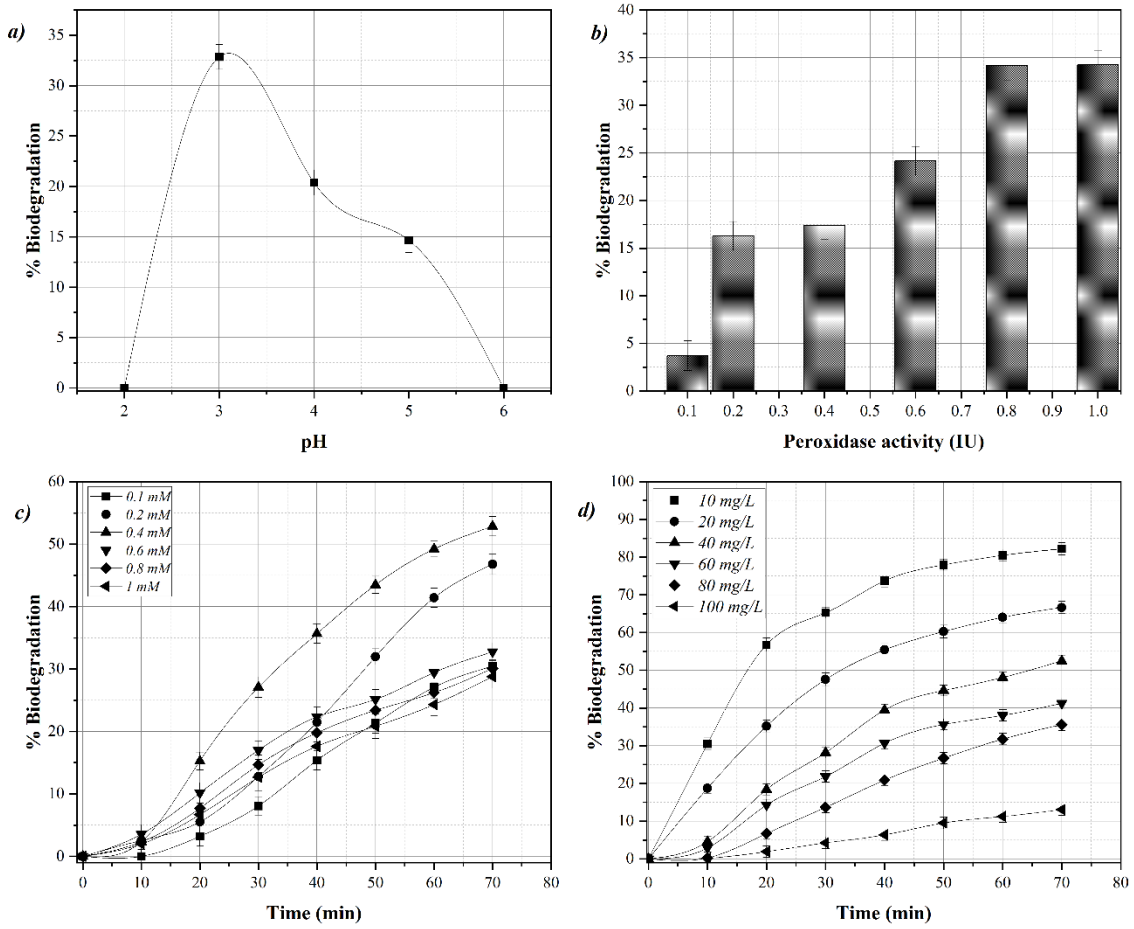

Figure 3. Optimization of process parameters for Acid Violet 109 biodegradation: a) pH optimization, b) enzyme concentration c) hydrogen peroxide concentration, d) dye concentration

2.5 Operational stability of cross-linked potato peel peroxidase for degradation of Acid Violet 109

The operational stability of CLEA potato peel peroxidase for degradation of anthraquinone dye was examined under the optimal conditions in several consecutive cycles. The results are given in Figure 4. In the second cycle, the cross-linked peroxidase kept 70.54 ± 1.58 % of the initial activiry, while in the third cycle, dramatical derecease to 19.66 ± 1.64 % was noted. Dahili et al. have studied the oxidation of 2,4-dichlorphenol by immobilized horseradish peroxidase. After 4 cycles, the immobilized peroxidase kept 30% of its initial activity [9]. Taking into consideration that this type of immobilization is founded on covalent bonds between the enzyme and the cross-linker without a carrier, the low mechanical stability of the cross-linked enzyme aggregates was expected.

3 Conclusion

In this study, peroxidase was isolated from an agro-industrial waste and used as cross-linked aggregates for biodegradation of the anthraquinone dye Acid Violet 109. Ammonium sulfate was the precipitating agent that showed best results. The cross-linker concentration of 0.1 % was optimal for peroxidase immobilization. At pH 3, with 0.8 IU peroxidase, 0.4 mM H_2O_2 and 10 mg/mL dye, biodegradation rate of 82.2 ± 1.65 % was achieved for 70 min. The enzyme kept 19.66 ± 1.64 % of its initial activity after the concecutive cycles of biodegradation.

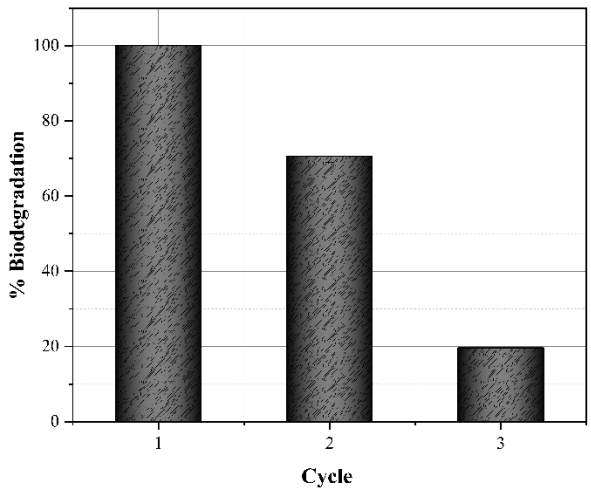

Figure 4. Operational stabillity of potato peel peroxidase for biodegradation of Acid Violet 109

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