Microfluidic technology for macro systems: removal of textile dyes from wastewater in a microreactor

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Abstract. Wastewater from textile industry contains considerable amount of dissolved dye that can trigger environmental issues if is not treated properly. Numerous methods have been developed to degrade recalcitrant pollutants safely and utterly. Among them, enzymatic treatment of wastewater is gaining attention due to the enzyme's specificity, easier manipulation, and generation of less toxic by-products. Still, the cost of enzymatic systems is the main limitation keeping the biocatalysts at lab-scale. Alternative solution for reducing the cost of enzymatic reaction systems is the use of microfluidic systems, which contribute to better mixing, process intensification and cleaner production. In this study, implementation of horseradish peroxidase for removal of the textile dye Acid Violet 109 is performed in a microfluidic reactor. The microreactor consists of three plunger pump units, two mixers and PTFE tube. The process parameters: residence time, dye, hydrogen peroxide, enzyme activity, the reactors' diameter and length were optimized. Under the optimal process conditions: 30 mg/L dye concentration, 0.8 U/mL horseradish peroxidase activity, 0.1 mM hydrogen peroxide, 0.25 mm reactor's diameter, 97,3 % removal was achieved at residence time of 6 min. The results from this study show that enzymatic microfluidic reactors are a convenient technology for dye removal.

1 Introduction

Treatment of wastewater is the most important mean to protect local ecosystems, by removing toxic compounds that cause disease in people, flora, fish, and wildlife. Numerous and diverse technologies are developed for purifying wastewater in order to accelerate the spontaneous cleaning of water [1]. There is neither single technique that is universal for every kind of wastewater, nor universal technique good enough to make wastewater from different sources potable. For example, wastewater from textile industry contains unused dyes from the dyeing process, heavy metals that are needed for the synthesis of dyes, such as mercury, chromium, cadmium, lead and arsenic [2]. If not treated, these chemicals end in water bodies. As a result, they impede photosynthesis by hindering light penetration and have negative effect on algae and the rest of the aquatic biota. Dyes that enter in fish and other living organisms are metabolized into toxic intermediates, effecting the fish and the organisms that consume fish [3]. If humans ingest fish that were exposed to that wastewater, the intestinal microflora will convert the dyes into toxic amino acids and cause severe health issues [4]. Furthermore, the same untreated wastewater is used by farmers in developing countries for irrigation of crops [5]. As a consequence, the soil quality and crop germination rates decrease. Although the textile industry has a big part in the worldwide economic growth –

China, European Union, India and United States as leading exporters of textile products [6], [7], with 10 000 synthetic dyes used in the industries [8–11], the removal of dyes and heavy metals from wastewater is of crucial importance.

Heavy metals can be removed by biosorbents [12], and the dyes can be degraded by different chemical, physical and biological methods. One of the trending methods is enzymatic degradation of dyes. Enzymatic treatments belong to 'green' processes due to the mild conditions required, and because enzymes themselves are generally recognized as safe reagents (GRAS). Different enzymes are used for degradation of dyes, such as peroxidases, lignin peroxidases and laccases, which can be obtained from different sources: plants and bacterial [13]. However, the cost of isolation and purification of enzymes limits their industrial use for wastewater treatment. Alternative solution for reducing the cost of enzymatic reaction systems is the use of continuous flow microfluidic systems. Continuous flow delivered by microtubular reactors offers several perks: high surface-to volume ratio; better mixing and mass transfer; laminar flow; process intensification and cleaner production which contributes to supporting the circular economy concept [14]. What makes microtubular reactors so attractive and desirable is the possibility of numbering-up, where the scale-up on industrial size can be easily done only with increasing the number of same reactors, and the size changes don't

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have impact on physical and chemical phenomena. Enzymatic treatments of coloured effluents are widely used. However, the existing literature dealing with enzymatic degradation of anthraquinone dyes in microtubular rectors is very scarce.

In this study, implementation of horseradish peroxidase for removal of the textile dye Acid Violet 109 (C.I. AV109) is performed in a microfluidic reactor. The microreactor consists of three plunger pump units, two mixers and PTFE tube. The process parameters: residence time, dye, hydrogen peroxide, enzyme activity, the reactors' diameter and length were optimized.

2 Materials and Methods

2.1 Materials

Horseradish peroxidase was obtained from Serva; C.I. Acid Violet 109 from DCC colorants (Ningbo); pyrogallol, monobasic potassium phosphate anhydrous, dibasic potassium phosphate trihydrate, sodium periodate from Sigma Aldrich, hydrogen peroxide 35% and sodium bicarbonate from Zorka Šabac; citric acid from Alkaloid; and trisodium citrate dihydrate from Alkaloid. All reagents were of analytical grade.

2.2 Methods

2.2.1 Enzyme activity assay.

The horseradish peroxidase activity was determined by pyrogallol as a standard substrate [15]. Briefly, the change in absorbance was monitored at 420 nm using UV-Vis spectrophotometer (Schimadzu 1800, Japan), for 3 min. The reaction mixture contained 0.013 M pyrogallol, 0.01 ml of 3 % v/v hydrogen peroxide and 0.01 ml enzyme solution in a total volume of 1.02 ml. One unit of activity is defined as the amount of peroxidase that will form 1 mg of purpurogallin from pyrogallol in 0.5 min at pH 7.0 and 20 °C.

2.2.2 Optimization of the reactor's diameter and length

The microreactor system consisted of three plunger pump units (LC-20AD XR, Shimadzu USA Manufacturing Inc) and two micromixers. The microreactor tube was made from PTFE. The output parameters – the flow rate of the reagents was kept in constant ratio 1:1:1, while the concentration flows were varied. One inlet was used for enzyme flow, other for the dye solution. These two flows mix in the first micromixer and then continue to the second micromixer where it is mixed with the hydrogen peroxide flow. Microreactors with diameters 0.25, 0.5 and 0.8 mm were studied in order to achieve maximal degradation of the dye. The degradation of the dye was monitored at 590 nm. For this optimization, the pH of the dye solution was 4.0, the enzyme activity 0.8 IU/mL and H_2O_2 0.1 mM. The reactor's length was studied by varying the

microtube length: 2.5, 6 25.5 m. It is important to note that that the initial dye concentration flow differs from the actual one, because there are three inlet flows. In all calculations, the actual concentration flow in the microreactor was taken in consideration, which is three times lower than the initial concentration flow.

2.2.3 Optimization of hydrogen peroxide concentration

The optimization of hydrogen peroxide was examined by varying its concentration in the range 0.025 – 0.1 mM. The dye AV109 was prepared by dissolving exact mass of dye in citric buffer 0.05 M, pH 4.0, leading to a final concentration of 30 mg/L. The flow rate of the reactants was kept at ratio 1:1:1. The enzyme activity was kept constant at 0.8 IU/mL. The experiments were performed at room temperature. The degradation efficiency was calculated by using the change in the absorbance that was measured at 590 nm.

2.2.4 Optimization of enzyme activity

The influence of the enzyme activity was examined by varying the concentration flow in the range of 0.01 – 0.8 IU/mL. The concentration of C.I. AV109 was 30 mg/L, the concentration of hydrogen peroxide 0.1 mM.. The flow rate of the reactants was kept at ratio 1:1:1. The reaction was performed at pH 4.0 and room temperature. The microtube used for this optimization was with 0.5 mm diameter and 6 m length. The residence time was 6.67 min. The reaction was monitored at 590 nm using UV-Vis spectrophotometer.

2.2.5 Optimization of dye concentration

The dye concentration was optimized by varying the initial concentration flow in the range 90 – 300 mg/L. The enzyme activity for this set of experiments was fixed at 0.8 IU/mL, the hydrogen peroxide concentration 0.1 mM. The actual dye concentration was 30 – 100 mg/L. The flow rate of the reactants was kept at ratio 1:1:1. The dye solutions were prepared in a citric buffer solution 0.05 mM, pH 4.0. The reaction was performed at room temperature. The microtube used for this optimization was with 0.5 mm diameter and 6 m length. The residence time was 6.67 min. The degradation efficiency was calculated by using the change in the absorbance that was measured at 590 nm. The dye AV109 was prepared by dissolving exact mass of dye in citric buffer pH 4.0. The flow rate of the reactants was kept at ratio 1:1:1. The enzyme activity was kept constant at 0.8 IU/mL. The experiments were performed at room temperature. The degradation efficiency was calculated by using the change in the absorbance that was measured at 590 nm.

3 Results and discussion

In this study, investigation of anthraquinone dye AV109 biodegradation by horseradish peroxidase in a

microreactor is performed. Previous research studies have shown that the optimal pH value for degradation of AV109 by peroxidase is 4 [16, 17].

3.1 Optimization of the reactor's diameter and length

Some of the most important characteristic parameters of a microreactor are its length and diameter. The surface area and the volume are derived from them, leading to the microreactor's productivity being dependent on the reactor's length and diameter. Herein, the effect of the reactor's diameter on dye removal is shown in Fig. 1. The reaction was monitored in microreactors with three different diameters: 0.25, 0.5 and 0.8 mm at residence time 11.67 min. As it can be seen in Fig 1, the reactor with 0.5 mm diameter showed highest dye removal – 80.23 ± 1.62 %. The other two reactors had slightly lower dye removal rate: 72.5 ± 1.84 and 66.82 ± 1.65 % for 0.25 and 0.8 mm diameter, respectively. Nonaka et al. concluded the importance of the diameter optimization in their study about condensation reaction in reactors with two different diameters: 0.5 and 4 mm [18]. They studied the asymmetric aldol addition reaction between p-nitrobenzaldehyde and cyclohexanone. For further experiments, microreactor with 0.5 mm was used.
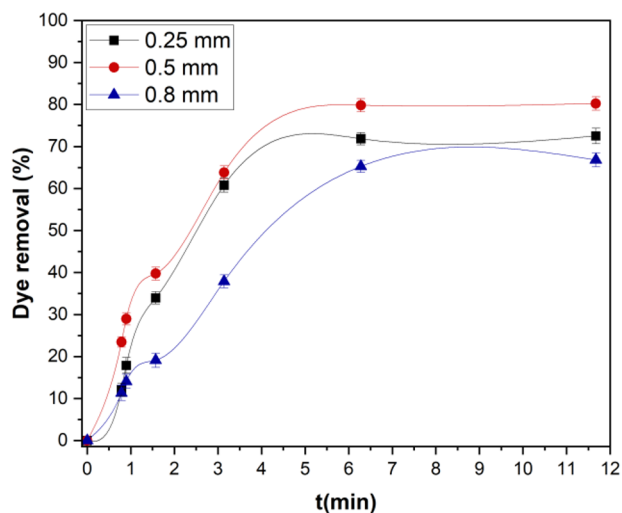

Fig. 1. Optimization of reactor's diameter. Conditions: pH 4.0, 30 mg/L dye concentration, 0.1 mM hydrogen peroxide, 0.4 IU/mL horseradish peroxidase activity, 6 m length, temperature 22 ºC.

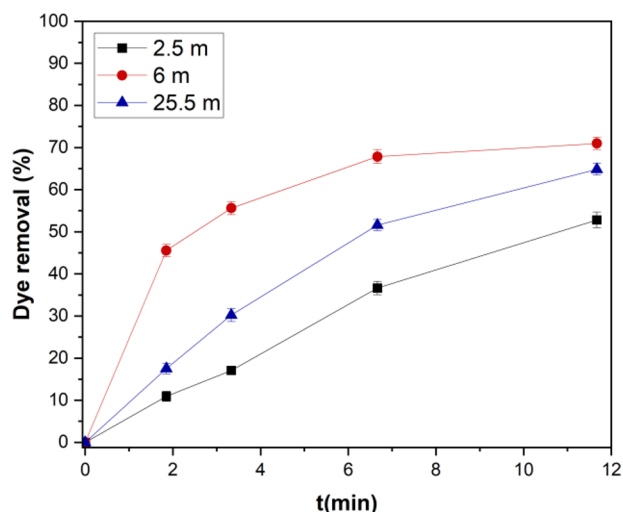Next, the influence of the microreactor's length was investigated.

Fig. 2. Optimization of reactor's length for AV109 removal by horseradish peroxidase. Conditions: pH 4.0, 30 mg/L dye concentration, 0.1 mM hydrogen peroxide, 0.4 IU/mL horseradish peroxidase activity, 0.5 mm diameter, temperature 22 ºC.

The degradation reaction was performed in reactors with 2.5, 6 and 25.5 m length and 0.5 mm diameter (Fig. 2). From Fig. 2, it can be concluded that reactor with 6 m length had shown best performance with 70.96 ± 1.68 % dye removal, while 52.81 ± 1.98 and 64.82 ± 1.53 % dye removal was achieved in a microreactors with length 2.5 and 25.5 m, respectively.

3.2 Optimization of hydrogen peroxide concentration

The hydrogen peroxide role in peroxidase catalysed reactions is to oxidize the enzyme and thereby convert it into its catalytically active form, while hydrogen peroxide is being reduced to water. Thusly, H_2O_2 can be considered as limiting reagent in the reaction, if its concentration is too low or as inhibitor if its concentration is too high. The influence of the hydrogen peroxide concentration is given in Fig. 3.
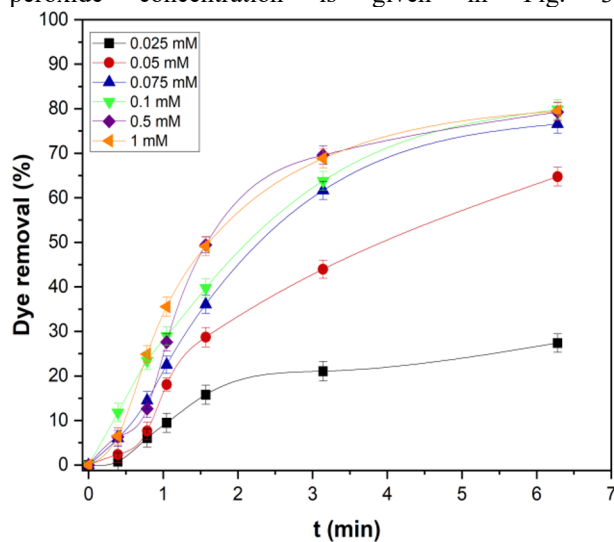

Fig. 3. Optimization of hydrogen peroxide concentration for AV109 removal by horseradish peroxidase. Conditions: pH

4.0, 30 mg/L dye concentration, 0.1 mM hydrogen peroxide, 0.4 IU/mL horseradish peroxidase activity, 6 m length, 0.5 mm diameter, temperature 22 ºC.

From Fig. 3 it can be seen that with increase of the H_2O_2 concentration, the dye removal increases up to a certain point where the concentration of H_2O_2 does not have any impact on the removal of AV109. Same findings were reported when peroxidase from soybean hull was used for degradation of anthraquinone dye in a microfluidic reactor [19, 20]. Similar results were achieved when peroxidase from potato peel is used for removal of AV109 in a continuous flow, where the biodegradation rate increases with the increase of hydrogen peroxide concentration. The positive effect of the microreactor's use can be observed in this optimization. When the degradation reaction had been optimized in a batch reactor, the inhibitory effect of hydrogen peroxide concentration was quite visible [21], where the biodegradation rate dropped from 57.35 ± 2.12 % with 0.1 mM H_2O_2 to somewhat below 40 % with 1 mM H_2O_2.

3.3 Optimization of enzyme activity

Enzymes are 'green' catalyst that operate under mild conditions. Peroxidase is an abundant enzyme that can be isolated from plenty of sources, such as horseradish, soybean, potato, cabbage etc. However, the time- and cost-consuming steps of enzyme purification dictates conscious use of peroxidase. In Fig. 4, the optimization of enzyme activity is shown, where the dye removal rate was investigated by varying the enzyme activity in the range $0.01 - 0.8$ IU/mL. It can be easily concluded that the highest dye removal of 97.32 ± 1.42 % was achieved with 0.8 IU/mL. Kalsoom et al. used higher peroxidase activity when they investigated the degradation of commercial textile dyes Drim Red CL4BN and Remazol Turquoise Blue 133 G, that are classified as reactive dyes [22]. The degradation was performed by peroxidase isolated from cauliflower leaves. They achieved 95% degradation of Remazol Turquoise Blue 133 G with 12 U/mL cauliflower leaves peroxidase (25 mg/L concentration, 0.8 mM hydrogen peroxide, 45 min of incubation time, at pH 3, 4, and 5). For the degradation of Drim Red CL4BN they used redox mediator *o*-diansidine hydrochloride. In 10 min, they achieved 97% decolorization with 15 U/mL peroxidase and 0.8 mM H_2O_2 at pH 2, without using the redox mediator. When o-diansidine hydrochloride is used, 12 U/mL is optimal activity of the cauliflower leaves peroxidase.
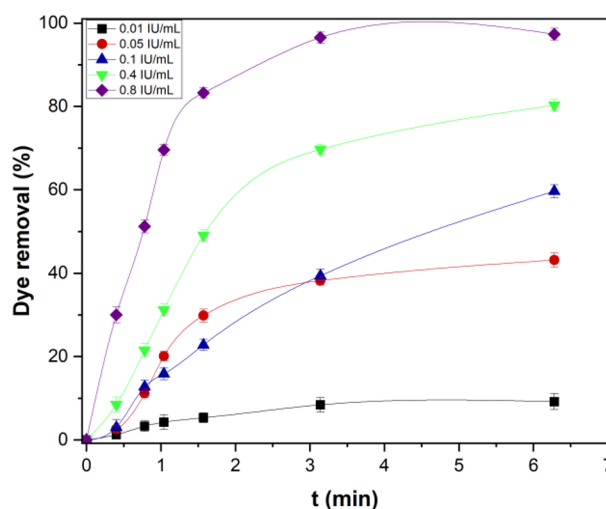

Fig. 4. Optimization of enzyme activity for AV109 removal by horseradish peroxidase. Conditions: pH 4.0, 30 mg/L dye concentration, 0.1 mM hydrogen peroxide, 6 m length, 0.5 mm diameter, temperature 22 ºC.

3.4 Optimization of dye concentration

Taking into account that the dye itself can act as an inhibitor and lower the efficiency of the process, the optimization of this parameter is necessary. The optimization was performed by varying the actual dye concentration in the range $30 - 100$ mg/L. The results are shown in Fig. 5. As it can be seen from the Fig. 5, with increase of the dye concentration, the dye removal rate decreases. The highest dye removal rate 93.21 ± 1.12 % was achieved when the concentration of AV109 was 30 mg/L, slightly dropping to 87.42 ± 1.02 % for 40 mg/L, followed by significant decrease to 42.37 ± 1.59 % when the dye concentration was 100 mg/L. Silva et al. investigated the degradation of the reactive dye Remazol Brilliant Blue R (RBBR) by soybean peroxidase [23]. They observed the RBBR degradation in the range $10 - 60$ mg/L. With 0.1 mM hydrogen peroxide, 70.4 U/mL peroxidase, 40 mg/L dye concentration, they managed to succeed 85.70 ± 0.44 % decolorization for 13 min. Here, it is interesting to observe that Silva et al. achieved 62.48 ± 1.01 % dye removal when the initial dye was 60 mg/L. At the same dye concentration, horseradish peroxidase in the microreactor system removed 78.13 ± 1.56 % of AV109.
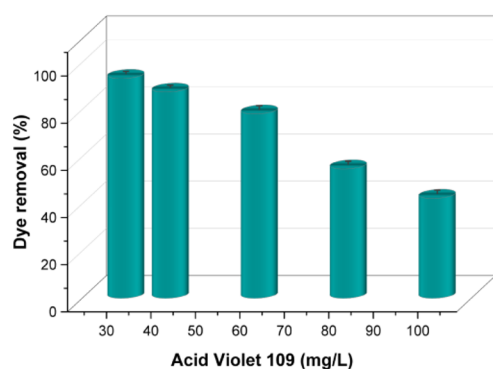
Fig. 5. Optimization of dye concentration. Conditions: pH 4.0, 0.8 IU/mL horseradish peroxidase, 0.1 mM hydrogen peroxide, 6 m length, 0.5 mm diameter, temperature 22 ºC.

The optimal parameters for the removal of C.I. AV109 by horseradish peroxidase in a microreactor are given in table 1.

Table 1. Summary of the optimal parameter values.

Parameter	Optimal value	Unit
pH	4	/
Residence time	6.67	m
Diameter	0.5	mm
Length	6	m
H_2O_2	0.1	mM
Enzyme activity	0.8	IU/mL
Dye concentration	30	mg/L
Dye removal efficiency	97.32 ± 1.42	%

4 Conclusion

In this study, the removal of the anthraquinone dye C.I. Acid Violet by horseradish peroxidase in a microreactor was investigated. The obtained results and the achieved removal efficiency show that this process has a great potential for commercial use, given the fact that by numbering up the microreactor, scale-up can be easily done.

References

1. A. Fahad, R. M. Saphira Mohamed, B. Radhi, M. Al-Sahari, Indian J. Sci. Technol. **12**, 25, 1–13 (2019)
2. K. Singha, P. Pandit, S. Maity, S. R. Sharma, *Green Chemistry for Sustainable Textiles* (Woodhead Publishing, 2021)
3. A. M. Elgarahy, K. Z. Elwakeel, S. H. Mohammad, G. A. Elshoubaky, Clean Eng. Technol. **4**, 100209 (2021)
4. J. Feng, C. E. Cerniglia, H. Chen, Front Biosci, **4**, 2, 568–86 (2012)
5. M. A. S. Jiku, A. Singha, M. Faruquee, M. A. Rahaman, M. A. Alam, M. Ehsanullah, Act. Ecol. Sin. **41**, 4, 358–364 (2021)
6. A. Atkar, M. Pabba, S. C. Sekhar, S. Sridhar, *Fundamentals of Natural Fibres and Textiles* (Elsevier, 2021)
7. O. M. Darwesh, S. S. Ali, I. A. Matter, T. Elsamahy, *Nanosensors and Nanodevices for Smart Multifunctional Textiles* (Elsevier, 2021)
8. R. Al-Tohamy, E.-R. Kenawy, J. Sun, S. S. Ali, Front Microbiol. **11**, (2020)
9. R. Al-Tohamy, S. S. Ali, F. Li, K. M. Okasha, Y. A.-G. Mahmoud, T. Elsamahy, H. Jiao, Y. Fu, J. Sun, Ecotoxicol Environ. Saf. **231**, 113160 (2022)
10. S. S. Ali, R. Al-Tohamy, E. Koutra, M. Kornaros, M. Khalil, T. Elsamahy, M. El-Shetehy, J. Sun, Biotechnol. Biofuels **14**, 1 (2021)
11. S. S. Ali, R. Al-Tohamy, J. Sun, Sci. Tot. Environ. **806**, 150665 (2022)
12. A. Ivanovska, B. Dojčinović, J. Lađarević, L. Pavun, D. Mijin, M. Kostić, M. Svetozarevic Adsorp. Sci. Technol. **2023**, 1 (2023)
13. A. Mishra, S. Takkar, N. C. Joshi, S. Shukla, K. Shukla, A. Singh, A. Manikonda, A. Varma Front. Microbiol. **12**, (2022)
14. T. Sokač Cvetnić, A. Šalić, M. Benković, T. Jurina, D.Valinger, J. Gajdoš Kljusurić, B. Zelić, J. Tušek, Catalysts, **13**, 708 (2023)
15. N. Ž. Šekuljica, N. Prlainović, A. B. Stefanović, M. G. Žuža,D. Z. Čičkarić,D. Ž. Mijin, Z. D. Knežević-Jugović, Sci. Worl. J. **2015**, 371625 (2015)
16. M. Svetozarević, N. Šekuljica, Z. Knežević-Jugović, D. Mijin, Mac. J. Chem. and Chemic. Engin. **39**, 196 (2020)
17. N. Ž. Šekuljica N. Ž. Prlainović, A. B. Stefanović, J. R. Jovanović, S. M. Jakovetić, Z. D. Knežević-Jugović, D. Ž. Mijin, Adv. Technol. **5**, 18 (2016)
18. S. Nonaka, H. Matsumoto, M. Nagao, Y. Hoshino, Y. Miura, React. Chem. Eng. 7, 55 (2022)
19. M. Svetozarević, N. Šekuljica, A. Onjia, N. Barač, M. Mihajlović, Z. Knežević-Jugović, D. Mijin, Environ. Technol. Innov. **26,** 102373 (2022)
20. M. Svetozarević, N. Šekuljica, A. Dajić, M. Mihajlović, Z. Popovski, D. Mijin, IOP Conf. Ser. Earth Environ. Sci. **1123**, 012005 (2022)
21. M. Svetozarević, N. Šekuljica, Z. Knežević-Jugović, D. Mijin, Environ. Technol. Innov. 21, 101289 (2021)

22. U. Kalsoom, H. N. Bhatti, K. Aftab, F. Amin, T. Jesionowski, M. Bilal, Bioprocess Biosyst. Eng. **46**, 3, 453–465 (2023)

23. M. C. Silva, J. A. Torres, L. R. Vasconcelos de Sá, P. M. B. Chagas, V. S. Ferreira-Leitão, A. D. Corrêa, J. Mol. Catal. B Enzym. **89**, 122–129, (2013)