Supporting information for

Amino-modified kraft lignin microspheres as support for enzyme immobilization

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Experimental section

Procedure for A-LMS synthesis

The procedure for A-LMS synthesis is described in study of Popovic *et al*.[1] Briefly, into a three-neck flask, the 0.5 g of lignin and 10 mL deionized water were added. Then, 2.0 g of PEI, 0.1 g of sodium dodecyl benzene sulfonate, and 10 mL of a sodium alginate emulsifier solution (5 or 10 wt %) were added into the flask. The reaction mixture stirred for 30 min and the temperature was elevated to 60 °C. The formed aqueous phase was added into 80 mL of liquid paraffin to form a suspension. Afterwards, 2.0 mL of epoxy chloropropane, cross-linker, was added and stirred. After 120 min, the reaction mixture is centrifuged, and the obtained solid was washed with both petroleum ether and ethanol and with water, by repeating sonication/centrifugation processes. A-LMS were obtained after freeze-drying for 24 h at −40 °C.

Characterization of A-LMS

Evaluation of A-LMS porosity

To evaluate **A-LMS** porosity the procedure earlier described elsewhere was used.[2] Following procedure, the 100 mg of dried **A-LMS** was completely wetted with absolute ethanol in a glass test tube. Density of material and **A-LMS**, Q_M and Q_P, and **A-LMS** porosity were calculated according to the Eq. S1-3

$$Q_M = {}^{m_M}\!/\!_{V - \left(\frac{m_W - m_M}{q_s}\right)} \quad \text{S1}$$

$$Q_P = {}^{m_M}\!/\!_{V} \quad \text{S2}$$

$$\varepsilon_P = {}^{Q_M - Q_P}\!/\!_{Q_P} = 1 - {}^{Q_P}\!/\!_{Q_M} \quad \text{S3}$$

Calculation is based on difference in weight and volume of dry and wet A-LMS. In Eq. S1 and S2 m_M represents weight of the dry **A-LMS**; m_w is the weight of the wet **A-LMS**; V represents volume of the wet **A-LMS** and q_s is the density of ethanol.

Determination of amino group content

Amino group content was determined via "back" (indirect) titration using 0.01 mol/L HCl for ultrasound treatment of A-LMS, standard 0.01 mol/L NaOH solution for titration, and phenolphthalein as indicator. The amount of amino group that reacted with HCl is determined based on difference of total amount of HCl used to treat A-LMS (20 mL) and the amount of HCl determined with NaOH titration.[3]

Determination of specific surface area of used A-LMS

The specific surface area of A-LMS_5 and A-LMS_10 was calculated according to the Brunauer-Emmett-Teller (BET) method from the linear part of the nitrogen adsorption–desorption isotherms (Fig. S1A and Fig.S2A). Nitrogen adsorption–desorption isotherms were determined using a Micromeritics ASAP 2020 instrument. Before analysis,

sample was degassed at 100 °C for 7 h under reduced pressure. The total pore volume (V_{tot}) was given at p/p_0 = 0.98. The volume of the mesopores was calculated according to the Barrett-Joyner-Halenda (BJH) method from the desorption branch of isotherm (Fig.S1B and Fig.S2B).

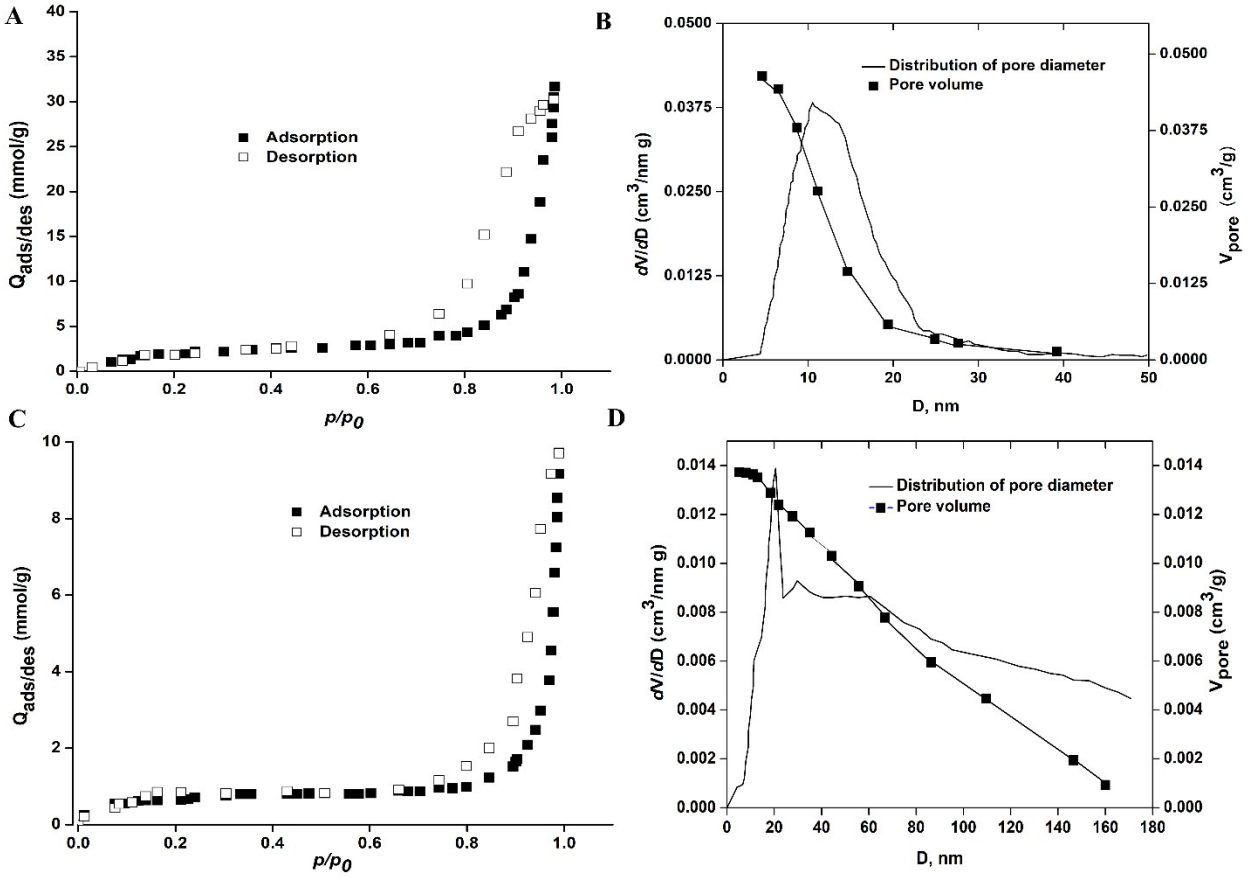


Figure ESI1. Nitrogen adsorption-desorption isotherms for A-LMS_5 (A) and for A-LMS_10 (C). Total pore volume and distribution of pore diameter relative to the pore volume for A-LMS_5 (B) and for A-LMS_10 (D).

Evaluation of A-LMS morphology by FESEM

The morphologies of the polymerized samples were observed by Tescan Mira 3 FEG field emission scanning electron microscopy (FESEM). A-LMS_5 was coated with Au before analysis using a Polaron SC502 sputter coater (Fig. S2).

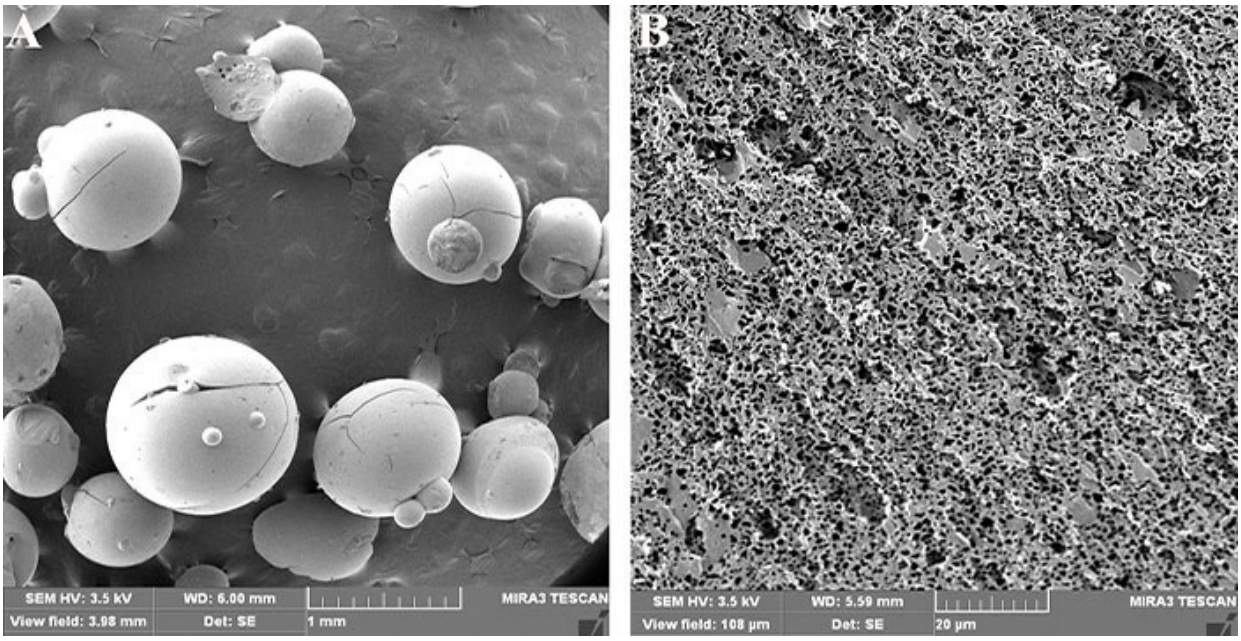


Figure ESI2. FESEM micrographs of A-LMS_5.

***β*-galactosidase-catalyzed GOS synthesis**

Samples were taken at different times from reaction mixtures with free and immobilized enzyme, and the reaction was stopped by heating samples at 100 °C for 10 min in order to inactivate the enzyme. Samples were ten-fold diluted with deionized water, centrifuged and then analyzed using HPLC.[4] Simultaneously, control samples (without enzyme) were prepared by exposure to the same temperature treatment, and the product was not detected in them.

HPLC analysis of samples

For quantitative analysis of obtained samples, the Dionex Ultimate 3000 Thermo Scientific (Waltham, USA) HPLC system was used. GOS determination was performed using a carbohydrate column (Hyper REZ XP Carbohydrate Ca^{2+}, 300 mm × 7.7 mm, 8 µm) at 80 °C. Deionized water was used as the mobile phase with an elution rate 0.6 ml/min during the analysis. Detection was performed by RI detector RefractoMax 520, (ERC, Germany). All data acquisition and processing was done using Chromeleon Software.

Lindane degradation (GC-MS method)

For monitoring of lindane degradation by free and immobilized laccase on A-LMS_5, GC-MS was used as described in our previosly reported study.[5] The hexane extracts of reaction mixtures samples taken at different time periods (1-6 days) during degradation of lindane with both free laccase and immobilized on A-LMS_5 were analyzed on GC-MS. The obtained results are compared to the result obtained during analyzis of heksane extract of control sample. The control sample was prepared by dissolving the γ-HCH standard in acetone, and then prepared γ-HCH solution in acetone (15 mM) was futher dissolved in Na-phosphate buffer (50 mM, pH 6.5) until final concentration of 30 µM.

In order to determine conformation ions for lindane presence, the full scan of the mass spectra (m/z ranging from 50 to 650) of control sample was done, and it was determined that the confirmation ions which are proving lindane presence are 111, 145, 183, 219 and 255 (m/z). These selected confirmation ions are in accordance with mass spectra for lindane stored in NIST library. The quantitative analysis of prepared control sample is carried out by using SIM mode (The selected ion monitoring mode) and as result lindane peak area (100 %) at the retention time of 6.4 min was obtained.

The identification criteria for lindane presence in all analyzed hexane extracts of reaction mixtures samples were retention time of 6.4 min and presence of selected comfirmation ions. The progress of lindane degradation with free and immobilized laccase on A-LMS were monitored by comparing the lindane peak area of the reaction mixture sample and of the control sample at the retention time of 6.4 min. The samples prepared with only A-LMS_5 and lindane were treated and analyzed as all the other samples taken from reaction mixtures with free or immobilized enzyme.

Parameters of GC-MS method

The injector and detector temperatures of the QP2010 GC-MS Shimadzu, Japan, were 250 °C and 280 °C, respectively. A 30 m x 0.25 µm ZB-5 Zebron, Phenomenex column was used. The initial column temperature of 100 °C, was maintained for 2 min. then increased linearly at 15 °C/min to 160 °C, maintained for 5 min, increased linearly at 5 °C/min to 270 °C and held for 2 min. The helium carrier gas flow rate was set to 1.08 ml/min and 1 µl of solution (lindane extracts diluted in hexane, 1:10) was injected. The retention time of lindane was 15.35 min. All extracts were analyzed in duplicate.

Results and discussion

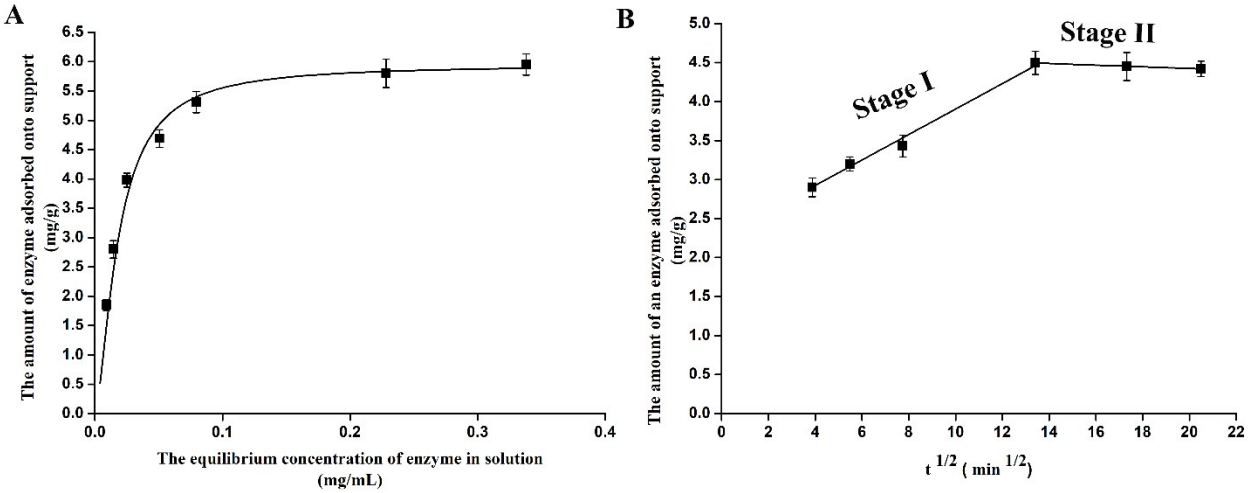


Figure ESI3. The Langmuir isotherm plot for the immobilization of β-galactosidase (A). Intra-particle diffusion model for β-galactosidase adsorption onto A-LMS_5 (B).

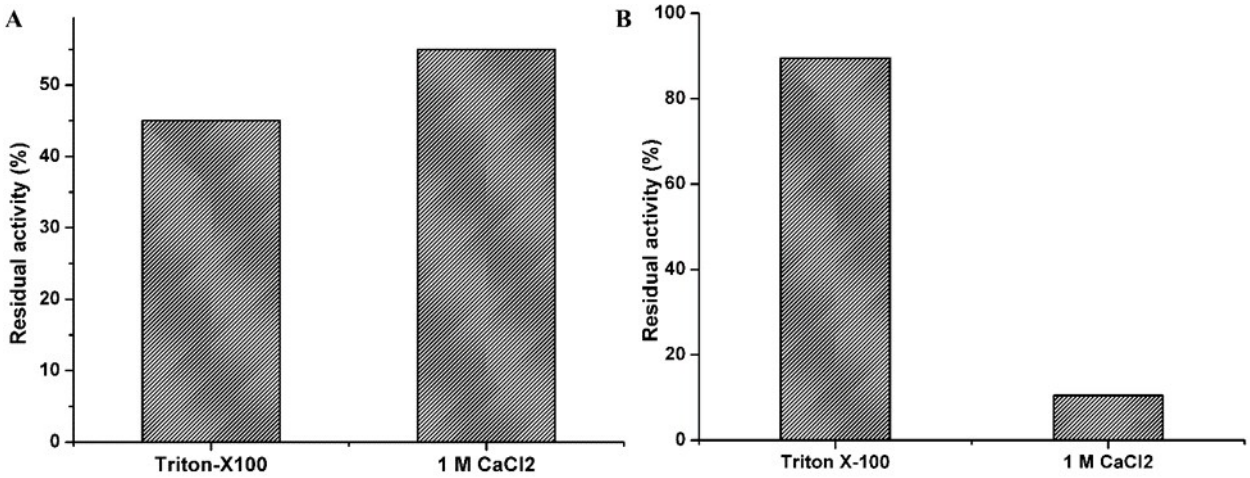
Table ESI1. Kinetic pseudo-first-order model parameters for β-galactosidase immobilized on A-LMS_5 at different offered protein concentrations (mg/g).

Offered protein concentration (mg/g)	k_1 ***(min^{-1})***	q_e ***(mg/g)***	R^2
2	0.0188	1.97	0.999
8	0.0169	2.95	0.999
15	0.0215	3.98	0.999
30	0.0241	4.49	0.999
45	0.0273	5.32	0.999
120	0.0350	5.77	0.999
175	0.0390	5.95	1.000

Table ESI2. The calculated parameters of the intra-particle diffusion model.

TYPE OF DIFFUSION	PARAMETER
BULK AND FILM DIFFUSION	R_1^2=0.986
	C_1=2.26
	k_{id1}=0.165
PORE DIFFUSION	R_2^2=0.992
	C_2=4.63
	k_{id2}=0.0107

Figure ESI4. Residual activity of laccase (A) and β-galactosidase (B) immobilized on A-LMS_5 after treatment with Triton X-100 and 1 M $CaCl_2$. The residual activity of immobilized preparation after treatment with Triton X-100 or 1 M $CaCl_2$ was calculated as activity after treatment relative to the activity of immobilized preparation before treatment, expressed in percent.



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