Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

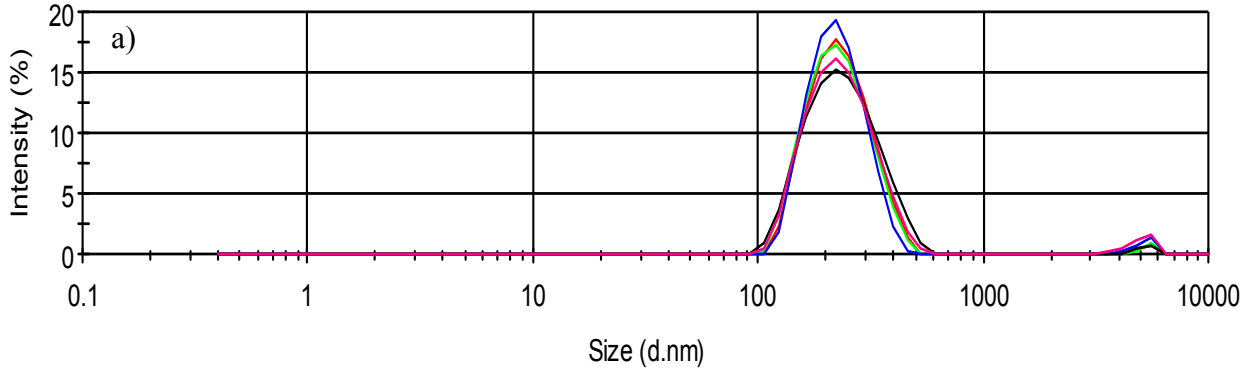
1. Immobilization yield

The BCA analysis allows to verify both amount and stability of the immobilized enzyme. Table S1 depicts the amount of protein assayed in the magnetically recovered solution and the wash water recovered from the three steps washing subsequent to immobilization. The amount of immobilized pectinase with prior EGDE activation was 0.28. After each step of washing, there was no measurable release of enzyme, implying stable immobilization.

In addition to indirect measurement of immobilized enzyme using equation 1, direct quantification of immobilized was carried out by injecting covalently immobilized Enz^{SP} into the BCA reagent. The maximum pectinase loading capacity of the NP^{SP} was 44 mg per gram of NP^{SP}. This result was equivalent to the maximum amount of immobilized pectinase obtained from the mass balance between the mass of protein in the initial and final immobilization solution (49 $\mu g_{Enz}/mg_{MNP}$).

2. Immobilized enzyme activity

Figure S2 represents the concentration of D-GalA produced as a result of the hydrolytic action of covalently immobilized pectinase on pectin in an STR. After 5 minutes reaction time, the reaction rate and the specific activity of the covalently immobilized enzyme were $10*10^{-5}$ M/min and $3\pm0.5*10^{-4}$ M/min*mg_{enzyme} respectively.



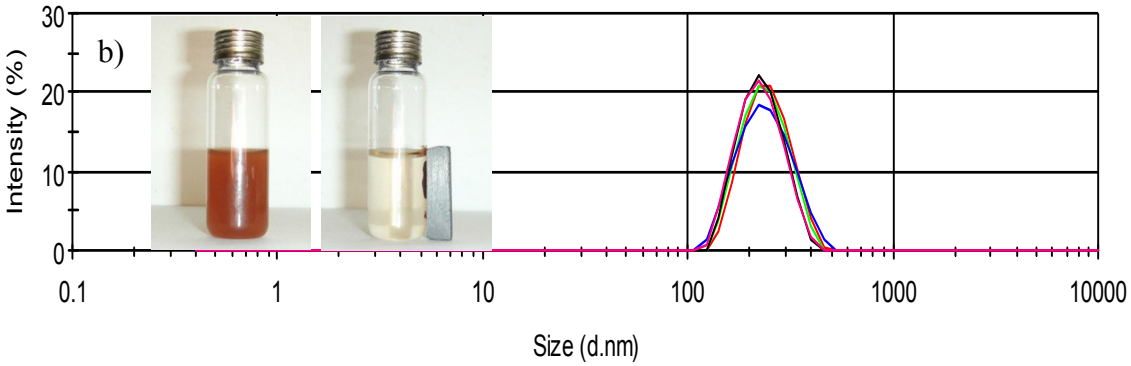


Figure S1: Analysis of particle size distribution before and after enzyme immobilization: a) DLS of NP^{SP}; b) DLS of Enz^{SP}

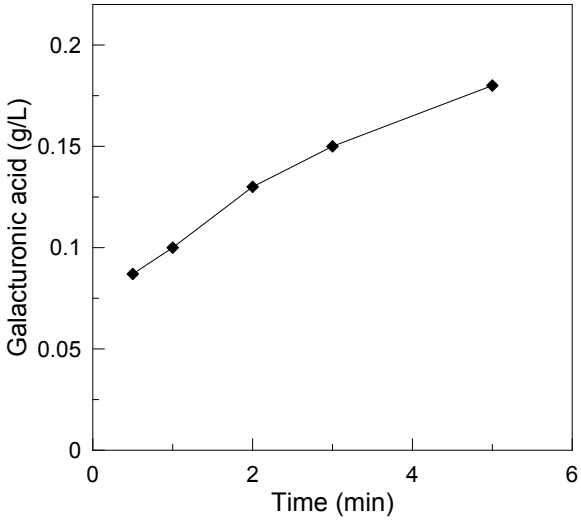


Figure S2: D-Galacturonic acid concentration versus time using NP^{SP} with covalently immobilized pectinase.

3. Evaluation of kinetic performance

The initial reaction rate (v_r) for free enzyme in a continuous flow reactor, assuming absence of enzyme-product inhibition due to simultaneous reaction and separation, is calculated based on the following mass balance equation [15]:

$$\frac{d(VC)}{dt} = (FC)_{in} - (FC)_{out} + v_r V_a \qquad 1$$

where F is the feed flowrate (L/s), C is concentration (g/L), v_r is the volumetric reaction rate (g/L s) and V_a is the reactor volume (L). At steady state, the accumulation term is zero and the reaction rate is:

$$v_r = \frac{F(C_f - C_p)}{V_a} \qquad 2$$

where C_f substrate concentration (g/L) in the feed and C_p is substrate concentration in the permeate (g/L). The BMR^{SP} is made of beads of catalytic bed supported by a flatsheet membrane, it can be assumed as a packed bed reactor (PBR). The actual reactor volume (V_a) is the fraction of the bed volume that is not filled with catalyst:

$$V_a = V_T - V_p \qquad 3$$

where, V_T is total bed volume (L) given by the BMR^{SP}'s geometric dimensions as:

$$V_T = H * A_m \qquad 4$$

where H is the catalytic bed thickness in dm, which is obtained from SEM micrograph, while A_m is the membrane area, which is 0.16 dm^2.

V_p is the volume of the beads of Enz^{SP} on the surface of the membrane calculated as:

$$V_p = \frac{m_p}{\rho_p} \qquad 5$$

Where m_p, total mass of Enz^{SP} (4.8 *10^{-3} g) deposited on the membrane and ρ_p is the density of ferrous particles (5150 g/L).

By combining equations 3 to 5, the volume of the reactor is then:

$$V_a = HA_m - \frac{m_p}{\rho_p} \qquad 6$$

By combining equations 2 and 6, the following equation is obtained for the volumetric reaction rate, v_r (mol/L h):

$$v_r = \left(\frac{F\rho_p}{H\rho_p A_m - m_p} * (C_f - C_p) \right) / M_w \qquad 7$$

where M_w is the molecular weight of the hydrolysis product, which is used to normalize the units of v_r to mol/L h.